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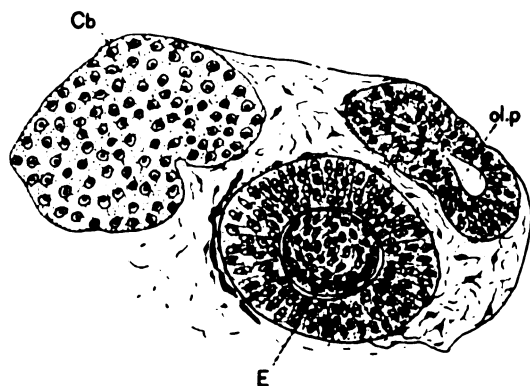
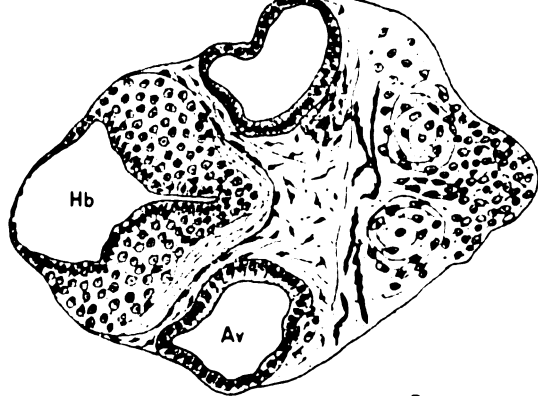
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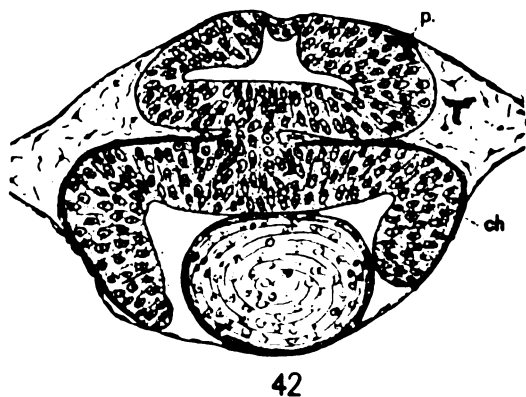
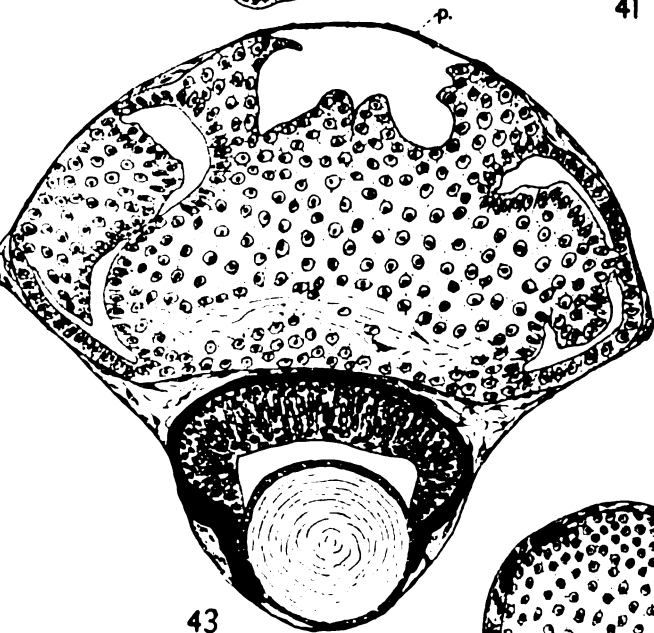
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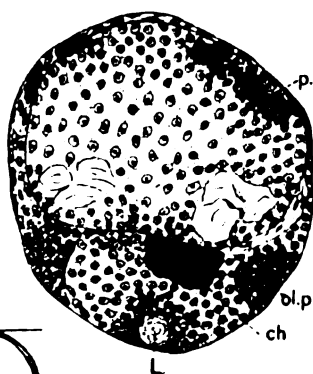


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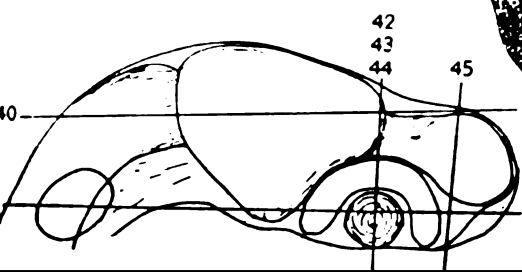


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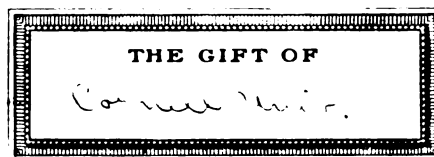
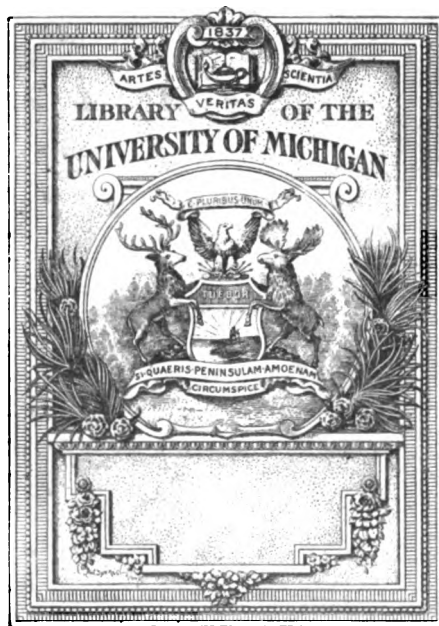


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ACIDOSIS AND ASSOCIATED CONDITIONS.*

JAMES EWING.

NEW YORK.

GENERAL STATEMENT.

The idea that the body may become poisoned by acid humors dates from the legendary days of medicine, but the conception of an intoxication referable to acid products of metabolism is a result of the rigid application of chemical laws to physiologic processes.

The theory of acid intoxication in its current form attributes to the acid action of principles arising from processes of metabolism a definite symptom-complex, occurring chiefly in diabetes, but seen in less striking form in many other diseases. For carnivorous animals it is easy to name the sources of such acid products of metabolism. They are found in the high phosphorus and sulphur content of the protein molecule, which, on oxidation, yields phosphoric and sulphuric acids. They appear in the long series of fatty acids which form the combustion-products of the fats. They arise also in the series of changes in carbohydrates from glycogen through glucose to lactic acid. From all these foodstuffs, especially the proteins and fats, the main tendency in metabolism is toward the production of acids. The exclusive flesh diet of carnivora increases this tendency; but the mixed diet adopted by man includes one prominent contrary factor in the rich alkalies of vegetable cells; while in herbivora the conditions are least favorable for the accumulation of acids in food metabolism.

The theory of acid intoxication regards only the chemical properties of these acids, and this property is to unite with alkalies. The sources of the alkalies which are available for the neutralization of acids are, first, those of the cells, and second, those of the blood and lymph; and it is the abstraction of the fixed alkalies of the organs and those of the blood which is believed to be responsible for the disorders of function and the symptoms of intoxication. Hence, it is the functions of the alkalies of the body which determine the symptoms of acid poisoning. Here it is obvious that vital processes are involved, for the function of respiration is dependent on the alkalescence of the blood; the solution

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of globulins in protoplasm is maintained by association with chemical bases; and the actions of ferments, digestive, circulating and intracellular, are determined by the reactions of their media. So that the theory of acid poisoning begins with the principles of normal food and tissue metabolism, recognizes in many conditions a mild grade of acid excess, which Naunyn called "acidosis," and finds its clearest expression in the acid intoxication of diabetic coma.

General chemistry, physiologic chemistry and, more recently, physical chemistry have all contributed essentially to our knowledge of acid intoxication, while the interpretation of the phenomena observed forms an important chapter in physiology. The clinical aspects of the subject, also, are among the most complex in general medicine, so that the development of the doctrine has been slow and difficult.

In a review of our knowledge of this topic, which is the object of these lectures, all the above departments require special consideration. It is proposed, therefore, reversing to some extent the order in which the knowledge was acquired, to present, first, the experimental basis of the doctrine of acid intoxication, and to consider the general problems as presented in the most pronounced form of acidosis, that of diabetes; second, to discuss the physiology and physiologic chemistry of the substances concerned; and third, to deal with the various diseases in which acidosis occurs, and determine, as far as may be, the significance of acidosis in such conditions as starvation, acute yellow atrophy, eclampsia, delayed chloroform poisoning, cyclic vomiting of children, gastrointestinal diseases and phosphorus poisoning.

I. EXPERIMENTAL BASIS OF ACID INTOXICATION— DIABETIC ACIDOSIS.

1. POISONING BY MINERAL ACIDS.

The first clue pointing to the occurrence of acid intoxication appeared in the work of Boussingault, who, in 1850, found that diabetic urines usually contain very large amounts of ammonia. To-day these results must stand as indications of excessive excretion of acids, but Boussingault's methods were attacked, and prevailing opinion, as stated by Nasse, and experimental results of Eylandt, Wilde, Gaethgens and others, indicated that administration of acids entailed no great loss of alkali in the urine and no serious disturbance of the alkalinity of the blood.

On the other hand, Miquel, by administering sulphuric acid by mouth in dogs, and Salkowski, by poisoning rabbits with taurin, which yields sulphuric acid in the body, demonstrated extensive loss of alkali in the

urine, while Lassar, by direct titration with acetic acid, found marked loss of alkalescence in the iced blood of various animals poisoned by acid.

The pathologic significance of the withdrawal of alkalies by administration of mineral acids seemed to be placed beyond doubt by the work of Walter, who showed that 0.9 gm. of hydrochloric acid by mouth per diem was constantly fatal for rabbits, and that the carbon dioxid content of the still faintly alkaline blood of such animals was greatly reduced. Organic acids, in his hands, were without comparable effect. Dogs failed to react to hydrochloric acid with toxic symptoms, and Walter's experiments show that this animal possesses an immunity against acid intoxication, protecting its blood alkalies by neutralizing 75 per cent. of the acids with ammonia. Since the symptoms in rabbits at the last stages of poisoning from 2 gm. of acid could be promptly relieved by intravenous injection of 0.5 gm. sodium carbonate, Walter concluded that the symptoms were referable solely to the withdrawal of alkali.

The symptoms of the acid poisoning appeared to be characteristic. The respiration was accelerated and the inspiratory movements were deeper and labored, while the blood pressure rose with tumultuous heart action. Later the dyspnea ceased, respiration failed, the heart became feeble and blood pressure fell. Walter was not impressed by the autopsy findings, except for gastric lesions produced by the acid, which he found difficult to avoid.

The study of the effects of administration of acids in animals has been pursued by many observers during the past thirty years, with the result of greatly extending and, in the main, confirming the conclusions of Walter, and these results have furnished the main experimental basis of the doctrine of acid intoxication. It has further been shown that rabbits may protect themselves to a certain extent by the formation of ammonia (Winterberg) and are less susceptible when on a protein diet. Eppinger was able to delay or prevent the symptoms in rabbits by feeding them on beef blood or egg albumin and by subcutaneous injections of amino-acids; while, after rectal injections of glyocol, he found that rabbits neutralized 75 per cent. of ingested acids by means of ammonia. (The contrary results obtained in this field by Pohl and Münzer have been set aside by Loewy.) On the other hand, starving dogs are just as susceptible to acids as are normal rabbits (Eppinger), and dogs readily succumb to the intravenous infusion of acids (Eppinger, Szili).

Typical acid intoxication in rabbits has been produced by phosphoric acid (Kobert) and by acid sodium phosphate (Spiro), but in dogs the acid effect was obscured by the action of the salt. Eppinger produced the usual symptoms of acid intoxication in rabbits by lactic acid.

All these results go to show that in dogs and rabbits there is a direct relation between the poisonous effects of acids and the acid-neutralizing resources of these animals, and that these resources may be altered almost at will by regulating the supply of alkali. The dog protects itself against acids readily because of its protein food, which furnishes an abundant supply of ammonia that may with safety be diverted from urea formation to neutralize the acids, while the rabbit, ordinarily without such source of ammonia, and compelled to call on the fixed alkalies for the neutralization of acids, may yet be supplied with ammonia by protein food. There appears to be no essential difference in metabolism between the two animals. Yet there is an important difference in the readiness with which these animals utilize proteins in the formation of ammonia, and this difference, Eppinger finds, depends on the size of the pancreas in the two animals. In the rabbit the pancreas is small, and whole proteins or their complex cleavage products, as polypeptids, are slowly broken up in the intestine, whereas glycocoll does not require further digestion, but may be readily absorbed and serve as a source of ammonia. A depancreatized dog Eppinger found to be more susceptible to acid poisoning than the rabbit (Szili). These facts may be of significance in connection with the pancreatic lesion of diabetes. According to this view, a simple quantitative deficiency in the digestive function of the pancreas may reduce the supply of absorbed amino-acids and the ammonia available for the neutralization of fatty acids and thus favor the development of acid coma.

ALKALESCENCE OF THE BLOOD IN ACID POISONING.

The explanation of the toxic effects of the withdrawal of alkalies from the blood and tissues is suggested by the diminished alkalescence of the blood and its lowered content in carbon dioxid. In fatal acid intoxication the blood may contain only 2 or 3 volumes per cent. of carbon dioxid as compared with the normal 28 to 40 per cent. (Walter), and Spiro found only 1.6 volumes per cent. in the blood of a dog receiving acid sodium phosphate. By direct titration Spiro observed in dogs a fall in blood alkalinity from 169 to 73 mg. sodium hydrate per 10 c.c. of blood. Szili infused 1/8N hydrochloric acid into the jugular veins of rabbits and dogs, 2 c.c. per minute. The rabbits survived about one hour and their blood alkali fell 78 per cent., while dogs succumbed in about thirty-five minutes and their blood alkali fell 75 per cent. In both animals the hydroxyl ion concentration fell 95 to 96 per cent. It is to be noted that in none of these experiments was the oxygen of the blood reduced.

These observations form the chief basis of the belief that acid intoxication kills by asphyxia of the tissue cells from accumulation in them of carbon dioxid. A partial dissent from this view is expressed by Loewy and Münzer, who believe that the production of carbon dioxid is diminished in acid intoxication; and this belief may be supported by the reduced consumption of many acid products of metabolism, resulting in lessened carbon dioxid formation, in diabetes. Beddard, Pembry and Spriggs found that the blood in diabetes is able to absorb a normal amount of carbon dioxid, even when its alkalinity and actual carbon dioxid content are greatly reduced. As a rule, the carbon dioxid tension of saliva and urine equals that of the secreting cells, but the above observers found that in diabetes the carbon dioxid tension of the urine is not increased. While the conception of cellular asphyxia is probably valid as far as it goes, further knowledge of the conditions determining the vitality of cells seems necessary before this theory can be regarded as proved.

Some other obscurities still surround the toxic action of acids. It has been surmised, but not satisfactorily proved, that vital organs are rendered seriously deficient in alkalies by administration of acids. An increased loss of alkali in the urine was demonstrated by Salkowski in taurin-poisoned rabbits, but he was unable to conclude that the loss was large enough to be serious. Schetelig found that the calcium oxid in the urine of acid-poisoned rabbits rose from 400 to 500 mg. per diem. From these and many other observations it is clear that acidosis is marked by considerable loss of alkali by the urine. But some of this urinary alkali is certainly diverted from the feces, which is the normal channel of 90 per cent. of the alkali excretion (von Noorden and Belgart).

Moreover, the body possesses practically inexhaustible supplies of alkaline earth in the bones, as well as very considerable resources in the food, and it still appears doubtful if these depots are not sufficiently available in acid intoxication to maintain the necessary alkali of vital tissues, while giving up those of the blood. The alkali content of such organs as the liver, kidneys and brain, in experimental acidosis, has apparently not been determined. It may be that the bone alkalies are not available in acute poisoning, in which alterations in these structures have not been found. In rabbits succumbing to large amounts of hydrochloric acid over a period of twelve days I could find no gross or microscopic changes in the bones. Yet in diabetes Frerichs states that the bones are abnormally light, and von Noorden concludes that the bone alkalies are extensively drawn on in diabetic acidosis. Gerhardt and Schlesinger

came to the same conclusion for severe diabetes, finding no other source for continuous loss of calcium in the urine. It is therefore probable that the bone salts are available in chronic acidosis, but not in the acute forms. On the other hand, the diseases associated with progressive loss of bone salts, rachitis and osteomalacia, have not declared themselves as forms of acid dyscrasia. There is a mass of clinical and experimental data indicating that the organs are rather susceptible to slight changes in their alkalies, but the pathologic significance of such changes in acid intoxication, especially in the acute and experimental forms, would be made clearer by accurate determination of the alkalies of the organs. According to Moore, the alkalinity of the tissues depends on interchanges of mono-sodium and disodium phosphates and by virtue of alterations in the relative amounts of these salts the cells may take up considerable quantities of acids without change in their reactions. Hence, Bainbridge supposes that by some interference with this protective mechanism of the phosphates and carbonates the tissues can no longer get rid of the acids produced and that their accumulation in the cells brings metabolism to an abrupt close. He regards it as extremely probable that the lack of some of these bases may disturb metabolism quite independently of the alkalescence of the tissues.

The method of estimating the alkalinity by the hydroxyl ion concentration has been applied to the blood by Szili and Benedict in Tangl's laboratory. They show that normal blood contains the same concentration of hydroxyl ions, that is, the same dynamic alkalinity, as distilled water. In animals poisoned by acid this concentration is reduced 95 per cent., so that this phase of alkalinity is practically obliterated. Physical and physiologic chemists will doubtless fail to agree on the significance of these observations, but they at least point to a new and interesting character of genuine acid intoxication, which may serve to separate true from spurious forms and thus prove a healthy control on the hasty application of the doctrine of acid intoxication. Landau holds that hydroxyl ions have little to do with blood alkalinity because they are derived from Na_2CO_3 and NaH_2PO_4 , salts which ionize very slightly.

An important phase of the question of blood alkalescence has been revealed by the work of Rzentkowski. It has long been realized that the acid-neutralizing power of the blood, its basicity, depends not merely on alkalies, but is a function of alkalies, proteins, urea and other nitrogenous substances. By alkalescence may be understood that part of the basicity due to alkalies. Rzentkowski titrated separately with N/20 sulphuric acid and litmus, the whole blood, the plasma, and the cells, with the following results:

Acid-neutralizing power of the blood in mg. NaOH per 100.

	Whole Blood.	Plasma.	Cells.
Total basicity	387	165	560
Basicity due to alkali	160.8	137.6	172
Basicity due to proteids	226	27.4	388

These results indicate a remarkable preponderance of acid-neutralizing power in the red cells. This conclusion is by no means new, but Rzentkowski found that 1 gm. of red cell protein is equal to 12 mg. of sodium hydrate in neutralizing acids, while 1 gm. of plasma protein equals only 3 mg. sodium hydrate, and, regarding the unit of blood, he shows that red cells may neutralize four and one-half times as much acid as the plasma. The red cells owe their basicity to proteins, the plasma chiefly to minerals, and loss of basicity in red cells may be due not to loss of protein, but to qualitative changes in the protein, such as the capacity to split off ammonia. Hence, the effects of acids in the blood are very imperfectly measured by the loss of alkali and probably also by the loss of carbon dioxide. It is to be considered, also, if these results do not lessen the significance of the data obtained by estimations of the hydroxyl ion concentration, for here is a type of basicity which is only distantly connected, if at all, with hydroxyl ions.

In any case, it appears that the adherents of the acid-intoxication theory have been relying on very inadequate methods of determining the acid-neutralizing power of the blood. Since this tissue must probably first feel the effects of any excess of acids produced in metabolism, it is quite unwarranted to assume diminished alkalescence of other organ cells until that of the blood has been definitely proved.

VISCERAL LESIONS IN EXPERIMENTAL ACIDOSIS.

The pathologic anatomy of experimental acid intoxication occupies a subordinate position among the data relating to this condition. Salkowski took occasion to note that the liver in his cases of taurin-poisoning was not fatty. Some gastrointestinal lesions have been referred to the direct effects of the ingested acid. Usually the visceral changes have been dismissed with the statement of the accepted fact that no alterations capable of explaining death were observable.

With the object of ascertaining if any of the gross or minute visceral lesions were comparable to those observed in forms of supposed acid intoxications occurring in man, I have studied the anatomic condition in rabbits after rapid and prolonged poisoning by hydrochloric acid. Subcutaneous injection of N/8 to N/4 hydrochloric acid were employed throughout. This method was chosen on account of the gastric lesions produced by administration by mouth and the uncertainty of absorption

of the acid. The intravenous route, while suitable for very rapid effects, is not adapted to more prolonged experiments. Weak acids are very irritating to the peritoneum. I was unable to inject fatal doses of acid beneath the skin without producing considerable damage to this tissue and eventually exciting an inflammatory process which retards absorption. Stronger solutions than N/8 usually cause necrosis and supuration.

A brief detail of some of these experiments follows:

RABBIT 1.—Black, male, weight 1,935 gm. Rapid subcutaneous administration of N/8 hydrochloric acid.

December 16: 12 m., 12 c.c. N/8 hydrochloric acid; 2:30 p. m., 12 c.c.; 5 p. m., 12 c.c. No symptoms.

December 17: 10 a. m., 12 c.c. N/8 hydrochloric acid; 12 m., 12 c.c.; 2 p. m., 12 c.c. N/4 hydrochloric acid; 3:30 p. m., 25 c.c.; 5 p. m., 25 c.c. Animal dull all day.

December 18: Animal died, 8:30 a. m.

Over a period of thirty-six hours the rabbit received, in divided doses, 60 c.c. N/8 hydrochloric acid and 60 c.c. N/4 hydrochloric acid. After the fifth injection the animal began to appear dull, and this symptom increased with the increasing strength of acid. No marked disturbance of respiration occurred. Death occurred fourteen hours after the last injection made at 6:30 p. m., at which time the animal did not appear to be seriously affected. Total acid received, 82 gm.

Autopsy.—Rigor, prompt, firm. Slight edema of subcutaneous tissue. Lungs very pale. Liver dark brown, no congestion or fatty degeneration. Kidneys appear normal. Stomach contains considerable food; shows no lesions. Intestine similar. Muscle pale. Bone marrow congested. Pia and brain appear normal.

RABBIT 2.—White, male, 1,720 gm.

December 18: Three injections, 12 c.c. each, N/6 hydrochloric acid. Rabbit appeared well; no symptoms.

December 19: Twelve injections, 12 c.c. each, N/6 hydrochloric acid. Rapid breathing noted after seventh injection. Later this became slow and shallow; ears cold, cyanotic; muscular twitching; very dull. Three hours after last injection animal much better.

December 20: Eating heartily. Red cells, 4,656,000; normal in appearance. Leucocytes, 7,500. Blood drop, cyanotic. Four hourly injections of 15 c.c. N/4 hydrochloric acid. Dull; head sways. Respiration, 48. One injection, 60 c.c. N/4 hydrochloric acid. Drowsy, cyanotic. Respiration unchanged. Thirty-five minutes after last injection, rabbit had a few general spasms and died. Total acid used, 2.16 gm. in fifty-two hours.

Autopsy.—Blood dark, fluid. Marked general edema at points of injection. Lungs, anemic; other viscera congested. No signs of fatty degeneration. Liver, dark red. Bile, light green. Muscles pale; marrow congested. Brain normal. Bladder contains 40 c.c. clear urine showing a trace of albumin; very many hyaline casts; ammonia nitrogen 4.2 mg. in 100 c.c. (Dr. P. A. Shaffer).

RABBIT 3.—White, male, 2,060 gm. For four days received 15 c.c. N/8 hydrochloric acid; then for five days 30 c.c.; on tenth and eleventh days 60 c.c. each. Died during night of eleventh day. During all this time the animal was increasingly dull, but no respiratory disturbance could be detected.

Autopsy.—Weight 1,750 gm., loss 310 gm. Abdominal wall showed extensive edema and in places slight purulent infiltration. Blood, fluid, dark. Lungs congested. Stomach well filled with food. Kidneys pale. Liver dark-colored, congested, not fatty. Bile brilliant green. Muscle very pale. Spleen soft, of normal size. Brain negative. Bones everywhere of usual appearance.

Conclusions.—From these experiments two conclusions seem to be obvious: first, that rabbits withstand considerable quantities of acids slowly administered (one receiving 1,485 gm. in 11 days); and, second, that when the acid is slowly given subcutaneously, drowsiness is the chief symptom, and the characteristic dyspnea can be recognized with great difficulty, if at all.

Summary of Gross Appearance of Organs.—On account of the venous character and fluidity of the blood and the congestion of the viscera one would conclude that the cause of death in these animals was some form of asphyxia, which is also indicated by the symptoms. Yet the anemia of the lungs, skin and muscles, while consistent with slow asphyxia, is not characteristic of any common form of slow asphyxia seen in man. The brilliant green color of the bile deserves more than routine notice, since it indicates a pronounced acidification of this secretion which may, perhaps, be regarded as pathognomonic of this form of acid intoxication. Otherwise I can only confirm the conclusions of others, that the organs show no gross lesions characteristic of acid poisoning.

Microscopic Examination.—Material fixed in formalin. Orth's and Zenker's fluids yielded the following general data: 1. An entire absence of fatty degeneration in frozen sections of liver, and kidney stained by Sudan III. 2. A peculiar granular and hydropic degeneration of the liver and, to a less extent, of the renal tubule cells. The eosin-staining material in the cells appeared to be very deficient. In some foci and in many isolated cells of the liver the cytoplasm was represented by a thin cell border and a few stainable clumps of granules. 3. Swelling and hyaline transformation of many voluntary muscle fibers. 4. Absence of any alteration in appearance of compact and cancellous bone tissue. 5. Marked chromatolysis of stichochrome cells of central nervous system.

This last character recalls similar changes reported by me in 1898, in a case of acute hydrochloric acid poisoning in an adult man. While none of these changes can be regarded as pathognomonic, and while their interpretation presents many difficulties, I believe that they constitute evidence which may prove of value and which can not wisely be ignored in the diagnosis of other forms of acid intoxication.

Mossé describes a microchemical test for acidosis. In fasting animals, in uremia, and after death from various poisons, he finds that the liver fixed in alcohol and stained by the May-Grünwald method or by neutral red, shows a distinct basophilic tint. Heiberg obtained a distinct reaction of this sort in the livers of mice fasting for seventy-two hours.

Mice fed five days on meat and butter and killed twelve hours after a single meal of carbohydrate showed normal staining reactions of the liver. I find that livers of rabbits poisoned by hydrochloric acid, fixed in alcohol, and stained by Nocht's method, or by eosin and methylene blue, stain densely blue and take little or no eosin; yet I doubt the wisdom of attaching much importance to slight variations in the staining reaction of material obtained at autopsy.

2. ACIDOSIS AFTER EXTIRPATION OF THE LIVER.

Another fundamental chapter in the rôle of ammonia in physiology and in the conception of acidosis concerns the relation of ammonia to urea-formation and to the functions of the liver.

It had previously been shown by Salkowski, Schmiedeberg and others that various ammonia salts administered to animals were not excreted as ammonia, but gave rise to increased excretion of urea and the conclusion appeared obvious that the ammonia was synthesized into urea. In 1886 Minkowski reported his remarkable observations on the influence on uric-acid formation of resection of the liver in geese, demonstrating that the synthesis of uric acid in birds is largely, if not exclusively, a function of the liver. The anatomic relations of the liver in birds permits the animals to survive, for about twenty hours, ligation of the portal vein, which diverts the blood to the inferior vena cava, the ligation of all arteries leading to the liver, and the partial or complete extirpation of this organ. In animals surviving this operation the excretion of nitrogen falls 50 to 60 per cent.; uric acid, the avian representative of urea, falls to one-thirtieth part of the normal excretion; the ammonia rises to 50 or 60 per cent. of the total nitrogen; while lactic acid, normally absent, reaches the enormous excretion of 3.5 gm. for twelve hours and constitutes one-half of the solid residue of the urine. The urine contains no sugar. The blood contains no sugar, but small quantities of leucin and tyrosin. On feeding these animals urea it was excreted as such, but amino-acids, glycocoll, asparagin, were largely broken up, with increase of ammonia, but partly excreted as such. That the excess of ammonia was not caused by its diversion to neutralize acid was shown by Engelmann, who, by giving alkalies, was able to reduce the ammonia without increasing the uric acid. The animals died in ten to twelve hours with vomiting, somnolence, coma, and occasionally with convulsions.

From these experiments several highly important conclusions could be drawn:

1. In birds the synthesis of uric acid (and urea) from ammonia is exclusively the function of the liver.

2. The appearance of increased ammonia in the urine may result from loss of the synthetic uric-acid-forming function of the liver.

3. The desamidation of amido-acids is possibly a function of the liver, but resides also in other tissues.

In order to determine how far the results in birds were applicable to higher mammals, direct observations on dogs were necessary. The partial or complete elimination of hepatic function in dogs has been accomplished by von Schroeder, by diverting the portal blood through a canula to a renal vein; by Hahn, Massen, Nencki and Pawlow, by means of an Eck fistula between portal vein and inferior cava, at times combined with ligation of the hepatic artery; and by Denys and Stube by injection into the bile ducts of acetic acid or by E. Pick with N/25 sulphuric acid. After all these procedures the animals rapidly passed into a state of intoxication marked by vomiting, thirst, somnolence, coma and convulsions. In animals with the Eck fistula the liver underwent fatty degeneration and atrophy, but collateral circulation was sometimes established and the animals survived. In these cases the symptoms were most striking, consisting of attacks of somnolence, muscular weakness and ataxia, followed by cerebral excitation, blindness, anesthesia and finally convulsions and coma. Feeding meat promptly induced an attack, sometimes fatal. The urine regularly showed a reduction in urea and excess of ammonia in combination with carbamic acid, and to the toxic action of this salt Nencki and Hahn chiefly attributed the symptoms. By the injection into normal dogs or by oral administration of this acid and its salts in animals with Eck fistulas they produced identical symptoms. In dogs with the Eck fistula the urinary ammonia nitrogen rose to 10 or 20 per cent., which is not very excessive; but it was noted that the ammonia increased rapidly with the appearance of toxic symptoms. In the brains of animals dead of this intoxication they found twice as much (20.5 per cent.) ammonia as in other organs (10.5 per cent.), and four times as much as in the brain of the normal dog (5 per cent.). Moreover, the arterial blood of a dog with an Eck fistula and toxic symptoms, contained as much ammonia as the portal blood of a normal dog.²

2. It may be noted here that carbamic acid (NH_2COOH) stands at the foot of the series of amido-acids $\text{C}_n\text{H}_{2n}(\text{NH}_2)\text{—COOH}$, and of the other amido-acids, as leucin, $\text{C}_6\text{H}_{12}\text{NH}_2\text{COOH}$, several are also present in these conditions. To what extent this or other acids of the series figure in the results of the Eck fistula and homologous conditions is not known. From this point of view it is possible to conceive of a special but in one sense spurious type of acidosis, constituted by the amido-acids, all of which involve combinations with ammonia.

In animals with the Eck fistula or in which the liver has been destroyed by injections of destructive agents in the bile ducts, the alkalinescence of the blood is not apparently much reduced. No very complete observations on this point are available, but Denys and Stube and E. Pick failed to find any marked reduction in the alkalinescence of the blood in their experiments. In this respect an important divergence exists from the conditions found in experimental intoxication by acids.

Salaskin and Zaleski find a striking difference in the results of extirpation of the liver from those following the Eck fistula or destruction of the liver by injection of acids into the bile ducts. With the Eck fistula the synthesis of ammonia into urea is defective; there is increase of ammonium carbamate in the blood and intoxication by this agent; the carbon dioxide of the blood is not reduced, and the urine is alkaline. After extirpation of the liver more complicated processes arise; the synthesis of ammonia into urea is defective; lactic and other acids are produced in excess with corresponding increase of ammonia; the carbon dioxide content of the blood is probably reduced and the urine is acid. Biedl and Winterberg were unable to verify some of the above observations, possibly owing to differences in technique, and, like Lieblein, they assert that ammonium carbamate is not directly concerned in these intoxications. While the exact details and interpretation of results may be left to future investigators, the significance of the observations of the Russian school need not be seriously doubted.

The studies in this field are of fundamental importance, not only for the physiology of nitrogenous metabolism, but for the doctrine of acid intoxication. They show that ammonia is essentially connected with the formation of urea and the functions of the liver, and that this urinary measure of acidosis may be increased by primary disturbance of hepatic function, in which an entirely secondary and theoretical acidosis exists.

When such a primary disturbance of the liver occurs, as from an Eck fistula, it is obvious that not only the urea-forming function, but all the other functions are disordered or abolished. Hence, the tendency to refer all the symptoms to the action of one agent, such as carbamic acid, must lead to grave error. It falls outside the scope of the present topic to consider in detail the biliary function, the relation to carbohydrate metabolism, and other phases of nitrogenous metabolism, or to dwell on the various symptoms accompanying disturbance of these functions, but it is probable that in hepatic disease associated with excess of urinary ammonia the influence of all these factors may have to be considered.

It has thus been shown by the foregoing lines of experimental research that:

1. It is possible to kill animals by injections of mineral acids or even of organic acids in large quantity, and such animals die with marked reduction of some of the acid-neutralizing properties of the blood and with diminished carbon dioxid content sufficient to explain their peculiar dyspnea. In such cases the urine shows marked reduction of urea nitrogen and corresponding excess of ammonia, which is diverted to neutralize the acids. The autopsy findings indicate death from asphyxia, and the organs are free from notable lesions.

2. When the functions of the liver are abolished, animals die with very pronounced nervous symptoms, extreme loss of urea nitrogen, high ammonia nitrogen, and the presence in blood and urine of carbamic acid and ammonia, the chief antecedents of urea, and with considerable lactic acid. The urinary signs of acidosis are pronounced and in many respects similar to those seen after experimental acid poisoning, but in the case of the Eck fistula no increased formation of acids has occurred, no abstraction of tissue alkali can be assumed, and death must result from some different mechanism, possibly from intoxication with accumulating antecedents of urea. The autopsy shows nearly complete destruction of the liver, secondary degeneration of other organs, but little reduction of blood alkalescence or carbon dioxid content. After extirpation of the liver more complicated processes arise, marked by increase of lactic acid and by excess of ammonia from failure of urea synthesis and probably from diversion of ammonia for the neutralization of acids.

Armed with these two striking pictures furnished by experimental pathology one may now safely enter the field of clinical medicine, to ascertain to what extent these prototypes are reproduced by spontaneous diseases of man and lower animals.

3. CLINICAL DEVELOPMENT OF THE DOCTRINE OF ACID INTOXICATION.

In 1857 Petters showed that the penetrating odor of the breath, urine and organs of diabetic subjects is caused by acetone, and he first referred the coma of diabetes to acetone poisoning. Later Kaulich demonstrated acetone in the urine of many diabetics and described a group of nervous symptoms as a characteristic clinical picture of acetonemia. This substance was subsequently found in the urine in many other conditions, some of which showed no nervous symptoms, and opinions became divided regarding the significance of acetonemia, some holding that it was the cause of the terminal intoxication of diabetes and of nervous

symptoms in many diseases, others believing that it possessed little or no important clinical significance.

Kussmaul was very direct in his criticism of the idea of acetone intoxication. He pointed out that acetone had long been used in the treatment of phthisis and that in considerable quantities it was entirely without toxic effects in man. In a case of advanced phthisis he administered 4 gm. daily for six weeks without noting any of the symptoms described by Kaulich. He tested the toxicity of acetone in rabbits and dogs and concluded that it was much less toxic than chloroform and slightly more so than alcohol, which it closely resembled in action.

Kussmaul's Coma.

In 1874 Kussmaul drew attention to the peculiar clinical features of certain cases of diabetic coma and sharply distinguished them from those of Kaulich's acetonemia. These features were:

1. A characteristic form of dyspnea. The respiratory movements were very deep, regular, *grosse Atmung*, and somewhat accelerated, 20 to 40 per minute. The movement of air was entirely unobstructed and venous congestion of the jugular veins during life and of the viscera after death was absent. The contrast between the general weakness and the deep, powerful respiration was most striking.

2. A rapid and weak heart action, 120 to 140 per minute.

3. Nervous excitation, restlessness, screaming, jactitation and hypogastric pain.

4. Coma, beginning one or more hours after the appearance of dyspnea and continuing till death. The temperature, slightly elevated at first, became subnormal and the pupils were normal or contracted.

Kussmaul concluded that this form of coma was caused by a direct stimulus of the respiratory center by some intoxication arising in the course of the disease and not from loss of oxygen, or accumulation of carbon dioxide, or from acetone poisoning.

Von Jaksch's Acetonemia.

The next advance in the study of diabetic coma was furnished by R. von Jaksch, who, in 1883, showed that Gerhardt's ferric chloric reaction in febrile and diabetic urines was referable to diacetic acid. Although, as Folin points out, von Jaksch was dealing with a mixture of diacetic and beta-oxybutyric acids, this was the first demonstration of acid substances in the acetone series. Instead of bringing the observation in line with the excessive urinary ammonia in diabetes, von Jaksch firmly believed in the direct toxic action of acetone and diacetic acid,

and he constructed an elaborate classification of the various clinical forms of acetonemia.

Another characteristic of diabetic urine which required explanation was the high proportion of ammonia demonstrated by Hallervorden. In one case he found 5.96 gm. of ammonia excreted in one day, an amount equal to 60 c.c. aqua ammonia and equivalent to 17.18 gm. of concentrated sulphuric acid. A parallel between the excretion of ammonia and the intensity of the disease appeared not to exist. Hallervorden accepted the view that the ammonia signified neutralization of acids and he administered 45 gm. of sodium bicarbonate with the idea of replacing the ammonia, but the experiment failed to prove the theory, as the ammonia remained unaltered.

Discovery of Oxybutyric Acid in Diabetes.

It was an essential contribution to the subject when Stadelmann, by balancing the known acids and bases, proved that diabetic urine contains a great excess of bases over that required to neutralize the inorganic acids, and hence that some unknown organic acid must exist in the highly acid diabetic urine. He attempted to isolate the acid and obtained a substance free from nitrogen and sulphur which he regarded as crotonic acid, $\text{CH}_2\text{CHCH}_2\text{COOH}$. He drew the further important conclusion that diabetic coma of Kussmaul's type is identical in character with that produced by Walter by ingestion of hydrochloric acid in rabbits, and that it must be, therefore, an acid intoxication, and he announced his determination to treat his next case of coma with sodium carbonate.

Stadelmann's work was promptly followed by the demonstration, simultaneously by Külz and by Minkowski, of beta-oxybutyric as the questionable acid in diabetic urine. Besides beta-oxybutyric acid and its derivative, diacetic acid, which may be regarded as of similar significance, diabetic urine is said to contain other organic acids which may figure in the intoxication. Reckoning acetone as diacetic acid, from which it is rapidly formed in the urine, the total quantity of diacetic acid in coma seldom reaches the maximum observed by Magnus-Levy—33 gm. in a case giving 120 gm. beta-oxybutyric acid. Usually acetone and diacetic acid are much less in amount, so that they usually contribute a very small part of the acidosis.

Traces of lactic acid have been found (Rumpf), but they may result from fermentation of sugar. The sarcolactic acid of Minkowski was probably a terminal or postmortem product. Volatile fatty acids were isolated by von Jaksch and by Rumpf, but Magnus-Levy criticizes the

methods employed and believes that such products are chiefly derived from changes in beta-oxybutyric acid. If present as such the quantities are trifling, so that diabetic acidosis is almost entirely due to diacetic and beta-oxybutyric acids.

While the majority of observers at this time were rather firmly convinced that the dyspneic coma of diabetes was the result of abstraction of alkalies from the blood and tissues, a true acid action, von Jaksch and others held to the theory of a "coma diaceticum" caused by direct toxic action of acetone compounds and it was generally acknowledged that decisive proof had not been furnished for either theory. It was especially the comparative resistance of carnivora to acid poisoning and the demonstration by Coranda that man resembles the dog in his feeble response to ingested acids that established a serious doubt, not yet entirely removed, that a fatal acid intoxication could ever arise spontaneously in the course of disease in man.

Clinical Studies of Oxybutyric Acid in Diabetes.

In the minds of many the final evidence in favor of the acid intoxication theory of diabetic coma has been furnished from the clinical side, whence alone it could come, by numerous quantitative estimations of the excretion of acetone compounds in a series of cases. On such observations in diabetic coma must rest the final verdict regarding the significance of this form of acidosis, for in no other conditions are the acetone compounds present in such quantity and nowhere else do characteristic symptoms seem so definitely connected with acidosis.

The earlier observations indicated that beta-oxybutyric acid or the sum total of acetone compounds in diabetes were formed in considerable but not in really large quantities. Stadelmann calculated from the excess of urinary bases that 8 to 23 gm. of the acid measured the daily excretion in his case of coma. Minkowski, after the administration of alkali, found 24 gm. on the day of fatal coma. Wolpe, in nine cases, found an average of 5 gm., but 23 gm. appeared in a comatose patient. He could not establish a parallel between the ammonia and acid excretion. Later Minkowski found that an excretion of 16 gm. daily had risen, after three weeks, to 53 gm. during coma. On the other hand, Baumann obtained only 10 gm. from 1½ liters of urine passed during the final forty-eight hours of coma.

The most extensive report on the quantitative excretion of beta-oxybutyric acid is that of Magnus-Levy, and many believe that the enormous quantities which this observer has found in certain cases has furnished the final proof of the acid intoxication theory of diabetic coma.

While beta-oxybutyric acid is absent in mild cases of diabetes, Magnus-Levy concludes from more than thirty subjects that this acid is present in all severe cases of the disease and tends to increase as the disease progresses, ranging in cases free from coma from 7 to 30 gm. daily. Yet he reports one severe case progressing unfavorably in which only traces were found, and his and other reports show great variations in the amount of acid excreted. In coma very much larger amounts appear, especially with alkali treatment. In one remarkable case (VI) the enormous quantity of 157 gm. of beta-oxybutyric and diacetic acids was estimated from the excess of bases over inorganic acids in 92 liters of urine. In another case 107 gm. were found, or 4 to 4.5 gm. per kilo of the patient's weight. Yet in four cases coma appeared when the excretion of acids ran from 20 to 40 gm. and one instance (II) coma developed when only 2.5 gm. were found.

It is clear that the very large quantities of acid recorded in these reports were largely referable to excessive diuresis from sodium bicarbonate. Yet the acid was present in the body, and Magnus-Levy calculates, with what validity is uncertain, that in a patient weighing 30 kg. and excreting 143 gm. of beta-oxybutyric acid, fatal acidification of the body would be produced by 80 to 150 gm. of the acid. However uncertain such computations may be, it is obvious that the quantities of organic acids in diabetic urine are far beyond any reasonable limit of safety.

The relation to symptoms must be determined, however, not solely from their presence in the urine, but from evidence of their existence and action in the body. From Magnus-Levy's table, considered merely as statistics, it is possible to argue that the coma was caused by glucose or ammonia, quite as much as by beta-oxybutyric acid, for all these substances were enormously increased by the alkaline diuretic. It is necessary to inquire to what extent the other features of Walter's acid poisoning are represented in diabetic coma.

The alkalescence of the blood, as measured by the carbon dioxide content, was found by Minkowski to be 30 volumes per cent. in normal human arterial blood, 1.17 volumes per cent. in a diabetic subject free from coma, and 3.3 volumes per cent. some weeks later during coma. At the same time the venous blood showed 19.5 volumes per cent. (1,000 mm. pressure). Kraus in thirteen cases found the carbon dioxide in venous blood (at 760 mm. pressure) from 10.20 to 19.77 volumes per cent. (normal, 35 to 40 volumes per cent.).

The titratable alkali of the venous blood was determined by Kraus in a fatal coma and found to have fallen to 125 mg. sodium hydrate for 100 c.c. of blood, normal 185 to 220 mg.

Magnus-Levy, using Löwy's method, found that normal blood yielded an alkali value almost never less than 260 mg. sodium hydrate, and seldom more than 400 mg., while this alkalinity was not greatly reduced in any other condition except diabetic coma. In mild cases of diabetes the alkalescence was found within normal limits. In three severe cases coma began with normal alkalinity (361, 290, 324 mg.), but fell during its progress to 234 and 124 mg. and in one case recovering under alkalies it rose from 324 to 362 mg., falling to 154 mg. in the blood five minutes postmortem.

These results with the carbon dioxid content and alkalinity of the blood, while far from demonstrative, may be regarded as consistent with the theory of acid intoxication.

In the blood and organs considerable quantities of beta-oxybutyric acid have been found which, especially in conjunction with other acids, may at least partially account for the reduced alkalescence. Minkowski determined the presence of over 0.22 per cent. of the acid in the blood of the cadaver. Magnus-Levy analyzed the blood and organs of three cases, finding as much as 0.22 per cent. in the blood, 0.14 per cent. in liver, 0.17 per cent. in spleen and 0.13 per cent. in the muscles, besides acetone, diacetic and lactic acids. In a patient of 45 kg. weight he calculated that the beta-oxybutyric acid amounted to 100 gm. Assuming an average fall in blood alkalescence in fatal coma of 220 mg. sodium hydrate (Löwy's method) he calculates that this could be produced by 572 mg. of beta-oxybutyric acid per 100 c.c. blood, which amount was actually found in the blood by Hogounenq.

ALKALI THERAPY IN DIABETIC COMA.

The results of alkali therapy further extend the parallel between diabetic coma and Walter's acid poisoning. The results of the practical test of Stadelmann's suggestion that the acid dyspneic coma might be combated by neutralizing the acids with alkalies were entirely unsuccessful, both in his own hands, with Hallervorden, who anticipated him, and with his immediate imitators. Patients in coma or excreting large amounts of ammonia failed to improve when given 20 to 40 gm. of sodium bicarbonate. When the quantity of alkali was increased better results were obtained. Magnus-Levy failed to abort fatal coma in three of four cases, although one of them received 200 gm. of sodium bicarbonate, but in a boy of 13 years he appeared to succeed in aborting two

attacks of coma by the enormous quantities of 210 and 200 gm. on the day of severest symptoms, although the urine remained acid. It is, of course, uncertain how severe these symptoms would have been without treatment.

In a second case, a child of 12 years, 100 gm. sodium bicarbonate was followed by the excretion of 7 liters of urine with 107.6 gm. of acid, and by marked improvement. Minkowski also reported one case of coma cured by alkali and Grube describes complete recovery from a definite coma in one adult with similar treatment.

In recent years reports and opinions regarding the efficacy of alkalies have varied greatly and failures certainly far outnumber the successes. Naunyn, in a recent review, states that in fully developed cases, intravenous injection of alkali does not relieve the coma, but at most causes temporary improvement. Before the outbreak of the coma, however, the results are much better. He was able to abort three attacks, but failed in the fourth occurring in the same patient.

The successful results seem to occur only in young subjects and in these, although some attacks may be influenced, later ones are fatal. Naunyn could report no case of coma in an adult cured by alkali. Although a consistent advocate of the alkali treatment, he freely admits that the good results may be due to other than the acid-neutralizing properties. In Young's case coma subsided for eleven days after venesection and injection with salt solution. Hilton-Fagge relieved another by infusion with the acid mixture sodium chlorate and sodium phosphate! In Naunyn's experience, 777 cases, the regulation of the diet with judicious use of carbohydrates seemed to be equally effective. Schwarz asserts that he cured by gluconic acid a case resisting alkali. Here the antiketogenic influence of this acid was supposed to come into action.

Finally it is admitted that dyspneic coma may develop during the administration of alkali and while the urine is alkaline, that is, when enough alkali is present to protect the tissues. In this connection it need not be overlooked that alkalies have themselves no inconsiderable tendency to increase the formation or excretion of acetone compounds.

THEORETICAL CONSIDERATIONS.

Are there any explanations which may account for these conflicting data and still leave the theory of acid intoxication a tenable hypothesis? Numerous efforts have been made to lighten the obscurities in this field with a success variously estimated by different authorities.

1. It may be maintained, first, that there are several types of coma in diabetes. Naunyn describes three, briefly indicated as: (a) Typical

acid dyspneic coma; (b) coma of doubtful type suggesting acid intoxication with severe acidosis; (c) coma of the same type without severe acidosis. One may, therefore, suppose that the cases which resist alkali are not the acid type of coma and that diabetic coma is not always of essentially the same origin. The majority of clinicians, however, are probably represented by von Noorden, who hesitates to accept this subdivision of the essential coma of diabetes. They prefer to suppose that the acid element of the coma is of necessity relieved by alkali, but that other contributing factors are uninfluenced.

2. Another line of argument is based on the possibility that the alkali when administered may not reach the acid in time or in sufficient quantity to neutralize it. A secondary hypothesis supposes that the acids before neutralization cause in the organs changes which themselves prove fatal, even after the acids are neutralized. The validity of these hypotheses can be estimated only by a minute consideration of the place of formation and the source of beta-oxybutyric acid, and it is not probable that in the present state of our knowledge a definite conclusion can be reached. Any acids derived from the fats and proteins of the blood or immediately reaching the blood from the tissues must be neutralized promptly by circulating alkali, when this is introduced intravenously, or even when administered by mouth, unless absorption is greatly retarded. It is not probable that acids from this source can cause any great loss of tissue alkali during alkaline treatment. Much the same condition must hold for the acids produced in the muscles.

Magnus-Levy conceives the acids as forming within organ cells and streaming into the blood plasma, but not before abstracting the fixed alkalies of the cells, which are thereby damaged, even in the presence of abundance of alkali in the blood. Hence, the alkali would be entirely effective only when present within the cells. From this quite hypothetical point of view it seems possible to explain the failure of the alkali therapy in the presence of a purely acid intoxication. In fact, while animals poisoned by acid *in extremis* react promptly to the injection of alkali it is a wonder that any one should expect the human body suffering from spontaneously forming intracellular acids to react in the same decisive manner.

Landau finds that in rabbits showing acidosis from phosphorus poisoning, administration of alkali has no effect on the total blood alkalescence, owing to the rapid excretion of the alkali by the urine, which becomes alkaline, and continuous formation of acids from toxic destruction of tissues. In fasting rabbits, in which the acidosis is referable to sulphuric and phosphoric acids and not to acetone compounds, sodium carbonate

promptly brings the blood alkalescence to normal and is able to hold it there, since in starvation acids are not so rapidly produced. Such considerations reveal some of the necessary limitations of alkali therapy in disease. At any rate, it must be accepted as a fact that diabetic coma is seldom or never cured by neutralization of acids, although it is probable that coma may be improved or even postponed by alkali therapy.

Against the purely acid nature of diabetic intoxication the objections seem to be far more direct.

1. There is the well-attested fact that coma closely resembling the dyspneic type may occur with little or no acidosis (Münzer and Strasser, Lépine, Kraus), but there seems to be insufficient ground for assuming that the condition is essentially different from that of the more typical cases. In Münzer and Strasser's case diacetic acid and acetone were present in abundance, but beta-oxybutyric acid was absent and no acetone compounds could be found in the blood of the cadaver. Magnus-Levy expresses the opinion that these observations are unreliable. In Magnus-Levy's Case 5, typical acid coma on one day was relieved by alkali, but recurred the next day without the dyspnea, yet Magnus-Levy believes that on the two days it was of identical origin.

2. Coma may improve when the urine still remains acid (Magnus-Levy) and may remain unaffected when the urine becomes alkaline.

3. The ammonia excretion, which is supposed to measure the degree of acidosis, does not run parallel with the quantity of acids as determined by other methods, and administration of alkali may not reduce the percentage of urinary ammonia.

It seems to be a still debatable question whether Kussmaul's dyspneic coma is specific of diabetes and acid poisoning.

Litten long ago described typical Kussmaul's coma in scarlatinal nephritis and, under the title "coma dyspepticum," he described very similar features in cases of marked gastric disturbance. Senator concluded that dyspneic coma occurs in other fatal diseases, as pernicious anemia and cancer, in which it is not associated with marked acidosis. Herzog also has recently pointed out the difficulties of distinguishing between diabetic and other forms of coma.

4. The ingestion or infusion of alkali has other prominent effects in addition to that of neutralizing acids. It is actively diuretic. In Magnus-Levy's most striking case of recovery from coma under alkali treatment, 220 gm. sodium bicarbonate in forty-eight hours increased the urine 273 per cent., the sugar 330 per cent., the organic acids 220 per cent. When the urine is increased from 2.6 liters to 7.1 liters it is

clear that opportunity has been given during this violent washing of tissues for excretion of many other noxious substances besides acids.

From the figures in this remarkable case taken without reference to the nature of the substance concerned we might suppose that the enormous output of sugar relieved the patient more than the removal of acids. Lépine believes that the alkaline treatment does good, not especially because it neutralizes acids—for the sodium salts of propionic, butyric, and lactic acids are toxic—but largely because it is diuretic.

It is on the physiologic action and not on the amounts of the substances excreted that the significance of the table depends.

Benedict adds to the obscurity of the subject by the results of observations on the hydrogen and hydroxyl ion content of the blood in diabetic coma. It was shown by Szili that the hydroxyl ion concentration of the blood, while identical with that of distilled water, is nevertheless reduced 95 per cent. in animals dying from injections of hydrochloric acid, in other words, that it is acid as determined by this method. Benedict, however, finds that in diabetes with severe acidosis the concentration of hydroxyl ions usually varies within normal limits. In coma it may be slightly diminished, but it is not far from the normal and is not lowered nearly as much as in other conditions in which symptoms of intoxication are absent. Farkas and Scipiades in two cases of normal pregnancy found lower readings for the hydroxyl ions than were obtained in Benedict's case of fatal diabetic coma. It may well prove, as Folin surmises, that physical chemical laws governing the solution of electrolytes are of little significance in pathologic physiology, and yet the fact remains that Benedict has brought to light a new point of difference between diabetic coma and experimental acid poisoning, and one that can not be lightly dismissed without further explanation.

5. The carbon dioxid content of the blood is greatly reduced in acid coma, but Meyer and Kraus find it greatly reduced in other conditions which are not marked by Kussmaul's signs, viz., in poisoning by many hemolytic agents, in miliary tuberculosis and erysipelas. Kraus also reports three cases of diabetic coma in which carbon dioxid content was very slightly altered, diacetic acid absent, and yet the symptoms could hardly be distinguished from dyspneic coma. These results suggest the need of repetition of the studies in the carbon dioxid content of the blood.

Finally it is a fact of frequent verification that patients may long withstand a severe acidosis, that coma frequently supervenes with diminishing excretion of sugar and ammonia, while Naunyn refers to a case which for six years withstood pronounced acidosis with the aid of alkalies, but finally died in coma in spite of them. All these observations seem

to indicate that there is some essential process which is responsible both for the acids and for the coma, and that a successful therapy must attack this earlier process.

In spite of the prodigious labor devoted to the subject over a period of thirty years, it is evident that the existence of an acid intoxication has not been placed beyond the field of debate. So many uncertainties still surround this question that nearly all of those who have carefully considered the available evidence have declined to take a positive stand on either side, and regard the matter as undetermined. The question is so complex and its phases reach out into so many branches of medical and collateral science as to suggest that the final verdict must await further progress in all departments. The practical value of the theory as a basis for hopeful therapeutics is its strongest plea for acceptance and here, at the same time, is presented its most potent weakness.

It is my personal opinion that the experimental basis of the theory of acid intoxication is sufficiently well founded and the chemical studies of diabetes furnish adequate grounds for concluding that acid intoxication produces coma in diabetes, but the clinical evidence is quite unsatisfactory. The clinical data point to the occurrence of identical symptoms in other conditions and even in diabetes in which there is comparatively little evidence of acidosis, while coma fails to develop with many diabetics with very severe acidosis. These facts appear to me irreconcilable with the assumption of an exclusively acid origin of diabetic coma, and lead me to think that acidosis is one of several factors leading to this coma, but one which stamps it with certain peculiar qualities.

The status of the whole subject of acid intoxication appears to depend on its position in diabetes. On this point Magnus-Levy is especially emphatic, asserting that in diabetic coma alone true acid intoxication exists; in all other conditions there is, in the sense of Naunyn, only physiologic acidosis.

All may not agree with this conclusion, but it is clear that in no other condition does the acidosis reach the same intensity as in diabetes. Yet the enormous amounts of beta-oxybutyric acid found by Magnus-Levy stand almost alone, and it is desirable that his results should be verified by independent observers.

It is equally clear that the direct attack on the problem by observations on human diabetes, on the results of alkali therapy, and on the experimental production of acidosis, have not succeeded in reaching a solution. More fundamental knowledge of the occurrence, source, chem-

istry, and physiology of the acetone compounds is needed before the correct interpretation of diabetic acidosis can be attained, and to the numerous contributions in this field attention may next be turned.

BIBLIOGRAPHY OF PART I.

- Bainbridge: *Lancet*, London, 1908, i, 911.
 Baumann, cit. by Magnus-Levy: *Arch. f. exper. Path. u. Pharmacol.*, 1899, xlii, 157.
 Beddard, Pembrey and Briggs: *Lancet*, London, 1903, i, 1366.
 Benedict: *Arch. f. d. ges. Physiol.*, 1906, cxv, 106.
 Biedl and Winterberg: *Arch. f. d. ges. Physiol.*, 1901, lxxxviii, 140.
 Boussingault: *Ztschr. f. prakt. Chem.*, 1850, ii, 281.
 Coranda: *Arch. f. exper. Path. u. Pharmacol.*, 1880, xii, 76.
 Denys and Stube: *Centralbl. f. allg. Path.*, 1893, iv, 102.
 Engelmann, cit. by Minkowski: *Ergebn. d. allg. Path.*, 1895, ii, 731.
 Eppinger: *Wien. klin. Wchnschr.*, 1906, xix, 111; *Ztschr. f. exper. Path. u. Therap.*, 1906, iii, 530.
 Ewing: *Arch. Neur. and Psycho-Path.*, 1898, i, 1.
 Eylandt: *Dissertation*, Dorpat, 1854, cited by Salkowski (l. c.).
 Farkas and Scipiadès: *Arch. f. d. ges. Physiol.*, 1903, xcvi, 577.
 Folin: *Tr. Assn. Am. Phys.*, 1907, xxii, 256.
 Frerichs: *Diabetes*, Berlin, 1884, A. Hirschwald.
 Gaethgens: *Centralbl. f. med. Wissensch.*, 1872, x, 833.
 Gerhardt and Schlesinger: *Arch. f. exper. Path. u. Pharmacol.*, 1899, xlii, 83.
 Grube: *Berl. klin. Wchnschr.*, 1904, xli, 915.
 Hahn, Massen, Nencki and Pawlow: *Arch. f. exper. Path. u. Pharmacol.*, 1893, xxxii, 161.
 Hallervorden: *Arch. f. exper. Path. u. Pharmacol.*, 1880, xii, 237.
 Heiberg: *Zentralbl. f. d. ges. Physiol. u. Path. d. Stoffwechs.*, 1907, ii, 721.
 Herzog: *Berl. klin. Wchnschr.*, 1899, xxxvi, 295.
 Hilton-Fagge: *Guy's Hosp. Rep.*, 1874, series 3, xix, 173.
 Hogounenq: *Rev. de méd.*, 1887, viii, 301.
 von Jaksch: *Ztschr. f. physiol. Chem.*, 1883, vii, 487; *Ztschr. f. klin. Med.*, 1886, xi, 307.
 Kaulich: *Prag. Vierteljahrsschr. f. prakt. Heilk.*, 1860, lxvii, 58.
 Kobert: *Schmidt's Jahrb. d. ges. Med.*, 1878, clxxix, 225.
 Kraus: *Ztschr. f. Heilk.*, 1889, x, 106; *Arch. f. exper. Path. u. Pharmacol.*, 1899, xxvi, 188; *Prag. med. Wchnschr.*, 1899, xxiv, 498.
 Külz: *Ztschr. f. Biol.*, 1884, xx, 165.
 Kussmaul: *Deutsch. Arch. f. klin. Med.*, 1874, xiv, 1.
 Landau: *Arch. f. exper. Path. u. Pharmacol.*, 1908, lviii, 207.
 Lassar: *Arch. f. ges. Physiol.*, Pflüger's, 1874, ix, 44.
 Lépine: *Rev. de méd.*, 1887, xii, 224; *Rev. de méd.*, 1888, viii, 1004.
 Lieblein: *Arch. f. exper. Path. u. Pharmacol.*, 1894, xxxiii, 318.
 Litten: *Ztschr. f. klin. Med.*, 1884, vii, 81.
 Löwy: *Arch. f. d. ges. Physiol.*, 1895, lviii, 462; *Centralbl. f. Physiol.*, 1906, xx, 511.
 Magnus-Levy: *Arch. f. exper. Path. u. Pharmacol.*, 1901, xlv, 389.
 Meyer: *Arch. f. exper. Path. u. Pharmacol.*, 1883, xvii, 304.
 Minkowski: *Arch. f. exper. Path. u. Pharmacol.*, 1884, xviii, 35; *Arch. f. exper. Path. u. Pharmacol.*, 1886, xxi, 41; *Mitt. a. d. med. Klinik zu Königsberg*, 1888, p. 174.
 Miquel: *Arch. f. Heilk.*, 1851, p. 479.

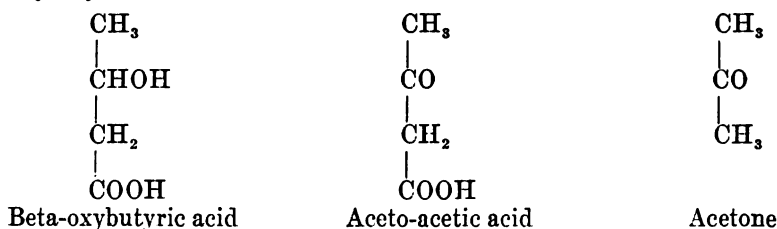
- Moore, cit. by Bainbridge: *Lancet*, London, 1908, i, 911.
 Mossé: *Ztschr. f. klin. Med.*, 1905, lx, 373.
 Münzer: *Deutsch. Arch. f. klin. Med.*, 1894, lii, 199.
 Münzer and Strasser: *Arch. f. exper. Path. u. Pharmacol.*, 1893, xxxii, 372.
 Nasse: *Wagner's Handbuch der Physiologie*, i, 137, cit. by Salkowski (l. c.).
 Naunyn: *Der Diabetes Mellitus*, ed., Vienna, 1908, Dr. Hölder.
 von Noorden: *Metabolism and Practical Medicine*, 1907, iii, 601.
 von Noorden and Belgart: *Berl. klin. Wehnschr.*, 1894, xxxi, 235.
 Petters: *Prag. Vierteljahrsschr. f. prakt. Heilk.*, 1857, lv, 81.
 Pick, E.: *Arch. f. exper. Path. u. Pharmacol.*, 1893, xxxii, 382.
 Pohl and Münzer: *Centralbl. f. Physiol.*, 1906, xx, 232.
 Rumpf: *Berl. klin. Wehnschr.*, 1895, xxxii, 669, 700.
 Rzentkowski: *Arch. f. exper. Path. u. Pharmacol.*, 1906, lv, 47.
 Salaskin and Zaleski: *Ztschr. f. physiol. Chem.*, 1902, xxxv, 246; 1900, xxix, 517.
 Salkowski: *Virchow's Arch. f. path. Anat.*, 1873, lviii, 1; *Ztschr. f. physiol. Chem.*, 1877, i, 1, 2.
 Schetelig: *Virchow's Arch. f. path. Anat.*, 1880, lxxxii, 437.
 Schmiedeberg: *Arch. f. exper. Path. u. Pharmacol.*, 1878, viii, 1.
 von Schroeder: *Arch. f. exper. Path. u. Pharmacol.*, 1885, xix, 381.
 Schwarz: *Prag. med. Wehnschr.*, 1901, xxvi, 361.
 Senator: *Ztschr. f. klin. Med.*, 1884, vii, 235.
 Spiro: *Beitr. z. chem. Physiol. u. Path.* (Hofmeister's), 1902, i, 269.
 Stadelmann: *Arch. f. exper. Path. u. Pharmacol.*, 1883, xvii, 419.
 Szili: *Arch. f. Physiol.*, 1903, cxv, 82.
 Walter: *Arch. f. exper. Path. u. Pharmacol.*, 1877, vii, 148.
 Wilde: *Dissertation*, Dorpat, 1855 (cit. by Salkowski).
 Winterberg: *Ztschr. f. physiol. Chem.*, 1898, xxv, 202.
 Young: *Brit. Med. Jour.*, 1903, i, 544.

II. *PHYSIOLOGIC CHEMISTRY OF ACETONE COMPOUNDS.*

The significance of acidosis must eventually be determined by the chemistry and physiology of the substances in question. The enormous quantities of acetone compounds found in diabetes are of much interest, but they may be of no more importance for the symptoms of the disease and no closer index of its nature than is the enormous excretion of sugar. We believe that sugar is neither directly nor indirectly a toxic agent in diabetes, but the doctrine of acid intoxication supposes that the acetone compounds are directly responsible for some of the most striking symptoms. Indeed, if this doctrine is accepted in its entirety most of the serious symptoms of the disease must be connected with these substances. As the direct study of diabetes has not succeeded in fully establishing the position of acid intoxication, new light must be sought in the chemistry and physiology of the acetone compounds and in the general occurrence of acidosis.

THE SOURCES OF ACETONE COMPOUNDS.

That a close relation exists between acetone, aceto-acetic and beta-oxybutyric acids is seen in their chemical constitution.



It is shown also by the readiness with which acetone may be derived from beta-oxybutyric acid in the test-tube, by the appearance of diacetic acid and acetone in the urine when beta-oxybutyric acid is administered to animals, and by the frequent association of all three acetone compounds in the urine.

Minkowski in 1869, distilling a dilute solution of beta-oxybutyric acid oxydized by potassium bichromate and sulphuric acid, obtained acetone, and he concluded that in man this acid was the forerunner of acetone. Diacetic acid is readily transformed into acetone at 100 degrees C. Minkowski fed beta-oxybutyric acid to a diabetic dog; Meyer gave it to healthy men, and Waldvogel and Magnus-Levy to diabetic patients, and in each case there was an increase of acetone or diacetic acid in the urine. These results have often been verified. Characteristic of the occurrence of acetone compounds is the fact that acetone, diacetic acid and finally beta-oxybutyric acid appear in order as the disease increases in severity and disappear in the reverse order as the disease improves. There is thus abundant evidence of the close relation between these three substances, justifying the use of the expression "acetone compounds," and indicating that they all have a common source in the body. Yet there are other modes of origin of acetone besides the direct derivation from oxybutyric acid, and several discrepancies exist between this theory of origin and the occurrence of acetone bodies in the urine, so that the exclusive origin of acetone from oxybutyric through diacetic acid can not be regarded as satisfactorily proven.

RELATION TO CARBOHYDRATES.

The old clinical impression that acetonuria resulted from fermentation of carbohydrates in the intestine was supported by the appearance of acetone in the test-tube when various carbohydrates were split up in the presence of alkalies (Fremy).

It has also seemed possible that the diabetic acetonuria may result from destruction of sugar or other carbohydrates of the body, since Harley found acetone and diacetic acid in the blood of dogs after injection of glucose and ligation of the ureters, and derived both those substances from sugar. Yet Pflüger regards the production of acetone from sugar in diabetes as far from proved, and it is obvious that Harley's experiments are not necessarily binding for diabetes.

That any considerable formation of acetone from carbohydrates occurs in human disease was rendered very unlikely by the discovery by Rosenfeld and Hirschfeld that carbohydrate-free diet induces marked acetonuria, and that in diabetes the withdrawal of carbohydrates may cause a large increase in acetone output which diminishes on the restoration of carbohydrates to the diet. On the other hand, in certain cases of diabetes in which the glycosuria has been reduced, the ingestion of carbohydrates increases both sugar and acetone (Waldvogel, p. 75). In diabetes and some other pathologic conditions the acetone of the urine greatly exceeds that of the breath, while in the dietetic acetonuria of normal subjects excretion by the breath usually exceeds that of the urine and may reach 70 per cent. of the total (Schwarz). It is a curious effect of carbohydrates that they tend to increase the proportion of acetone eliminated by the breath and to decrease that of the urine, thus transforming the pathologic into a so-called physiologic condition.

Carbohydrates vary in their capacity to influence acidosis. In starvation Jorns asserts that he has observed that starch increased the acetone, but that saccharose, and, to a much less extent, glucose decreased the total acetone excretion, while glyconic acid diminished the acetone of the breath while increasing that of the urine. The striking benefit in diabetes obtained by von Noorden and others, and recently by Herrick, from the use of oatmeal and other carbohydrates, suggests that the diabetic organs may lose the capacity to burn one carbohydrate while retaining command over others. Yet the results of the oatmeal diet in diabetes have been very conflicting, and the exact cause of the improvement in such cases is not certainly known.

The marked antiketogenic influence of levulose noted by Möhr and Loeb has also been applied in the treatment of diabetes. According to Hirschfeld, at least 80 gm. of carbohydrate must be taken daily to prevent the occurrence of dietetic acetonuria, but the observations are hardly sufficient to establish any definite rule.

Two hypotheses have been suggested to account for these observations: 1, that the burning of carbohydrates facilitates the combustion of acetone

compounds; and, 2, that the presence of carbohydrates spares the body fats and thereby stops the formation of acetone compounds.

Waldvogel is the chief exponent of the view that carbohydrates merely prevent the burning of fats, and in a detailed consideration of various forms of acidosis he was able to show rather clearly that acidosis usually begins with the burning of body fats. In phloridzinized dog acidosis begins after the liver has consumed its glycogen and has begun to call on the fats and proteins for energy, and here feeding protein as well as carbohydrate reduces the acidosis (Baer, Blum). It thus appears that in dogs acidosis will not arise as long as there are available carbohydrates anywhere in the body.

Yet this fact does not prove for human acidosis that the carbohydrates merely replace the fat, although it is true that carbohydrates are more readily burned in health than are fats. If the carbohydrates merely replaced fat, then there should be a quantitative relation between the amount of sugar needed and the degree of acidosis (fat consumption) to be combatted. But, as von Noorden shows, no such relation exists, since 100 gm. of glucose, equivalent to 30 to 35 gm. of fat, promptly obliterates most traces of acidosis after meat fat diet, and the acidosis does not recur when muscular exertion raises the consumption of fat, as he supposes, above its former level. Satta shows that comparatively small amounts of carbohydrate suffice to prevent acidosis, while much larger quantities are needed to reduce it when once established. Hence it appears that the burning of carbohydrates facilitates the normal and complete combustion of fats, as though the oxidation, more readily established in carbohydrates, was communicated in some way to the fats. Möhr supposes that the carbohydrates, which are rich in oxygen, yield nascent oxygen within the cells and thus directly consume the fats, but there appears to be no definite chemical ground for this opinion. Whether the carbohydrate prevents the formation of acetone compounds or facilitates their combustion when formed is not known.

According to Satta, the formation of acetone compounds is the result of a specific disturbance of fat combustion resulting, like the excessive formation of ammonia and increase of uric acid, from injury of organ cells. In pneumonia acetone production continues in spite of added carbohydrates, increases with the fever, and subsides when the general condition improves. Möhr traces this result to the slower combustion of carbohydrates. In diabetes Möhr and others believe that there is a qualitative disturbance in fat combustion connected with the failure to utilize carbohydrates, so that from a given quantity of fatty acids more acetone compounds result than in normal metabolism.

It was thought that further light on this subject might be secured by testing the effects on acidosis of rectal and subcutaneous injection of sugar. J. Muller, Schumann-Le Clercq and Waldvogel got no influence from sugar so administered, but Satta secured results as good as with administration by mouth.

While these and other related questions still await solution, at present it appears that in addition to fat and protein sparing action, a certain amount of carbohydrate combustion is necessary for the normal processes of metabolism and that when the carbohydrate is deficient metabolism is disturbed in various ways, one of which is an abnormal and incomplete combustion of fats. An interesting suggestion in this much debated field comes from Packard, who finds that *Fundulus* embryos, maltose, levulose and glucose increase resistance to lack of oxygen, acting, according to this observer, as depolarizers in the process of protoplasmic respiration and enabling this process to go on to some extent in spite of the lack of oxygen.

RELATION OF ACETONE COMPOUNDS TO FATS.

While the final proof that acetone compounds may be derived from fats in the test-tube has been reserved for recent workers, it was shown by Cotton that fats on oxidation yield acetone, and it was partially proved by Schreiber that beta-oxybutyric acid appears when butter is warmed with potassium permanganate and alkali. The butter, however, contained traces of protein, as well as butyric acid.

Geelmuyden first observed a marked increase of acetone in healthy men fed on butter, and his observations formed the beginning of the present extensive clinical data by which the origin of acetone from fats has been proven. Having in mind the fact that acetone excretion in many cases is not accompanied by excessive destruction of proteins, and accepting the clear indication that acetone is not derived from carbohydrates, Waldvogel saw no other source for the acetone compounds than the fats, and with Hagenberg he demonstrated a definite increase in acetonuria by feeding olive oil to fasting subjects and diabetics, and by adding butter and olive oil to full mixed diet of normal subjects. Many exceptions to this ketogenic action of fat were found to be due to personal peculiarities of the subject, to the amount and character of the food in the intestine, and especially to the readiness with which the fats were absorbed.

In these earlier experiments much confusion arose from failure to take account of the antiketogenic effects of the carbohydrates. It was found that the increase of acetone in patients on mixed diet was not

constant and never marked, seldom exceeding 100 mg. per diem. One of the disturbing factors was shown by Geelmuyden to be the carbohydrates of the food, while Schwarz found that administration of 150 gm. of glucose caused a prompt disappearance of acetonuria in a patient who showed acidosis from a meat-fat diet. On such a diet free from carbohydrates more pronounced grades of acidosis occur. Gerhardt and Schlesinger each subsisted on meat and fat for eight days. Acetonuria appeared on the second day, diacetic acid on the third day, and beta-oxybutyric acid in considerable amounts on the fifth and seventh days. Yet even here the presence of protein in the food diminishes the ketoplastic action of fat, since acetonuria increases as the albumin of the food is decreased (Rosenfeld). This result may be referred to the action of carbohydrate radicles in protein and to its general fat-sparing influence (Geelmuyden). Hence it appears that the ketogenic influence of fats can be satisfactorily studied only in fasting subjects.

A third disturbing factor of importance was pointed out by Joslin in the variable absorption of fats, a source of error which previous observers had largely overlooked. It was found by Hirschfeld that, of the components of fat, glycerin is strongly antiketogenic, and Hagenburg and Joslin showed that neutral fats are not ketogenic, probably because of their glycerin content, and that the ketoplastic action of fats increases with their content of free fatty acids. The failure of palmitic and stearic acids to increase acetone, as noted by Schwarz, Joslin attributes to their failure to be absorbed, while oleic acid which is readily absorbed is markedly ketoplastic. Butyric acid he found inert in a healthy fasting subject.

In diabetics various forms of fats have been found to increase acidosis, as butter, olive oil, bacon, cream. Sodium butyrate greatly increased the acetone in a case of Schwarz. Yet Magnus-Levy gave a mild diabetic 20 gm. of beta-oxybutyric acid in three hours and the urine failed to yield any acetone compounds. Loeb and Möhr found a general parallel between the amount of fats taken by diabetics and the excretion of beta-oxybutyric acid. On giving a diabetic 50 gm. of water-free butyric acid there was an increase in the beta-oxybutyric acid excreted of 19.5 gm.

It is obvious that the above observations have a practical bearing both on the regulation of diet in acidosis and on the interpretation of the signs of acidosis. The lower fatty acids, butyric, caproic and isovaleric, are so readily converted into beta-oxybutyric and yield relatively such large amounts, as shown by Schwarz, that it seems highly desirable that these acids should be excluded from diabetic diet.

According to Schwarz, the ketogenic action of fats in severe diabetes is very little influenced by carbohydrates and increases with the loss of the capacity of the body to burn carbohydrates and fats. In a case of diabetes, in which the patient received 200 gm. of beef fat daily for three days, Schwarz saw dyspneic coma develop, and Waldvogel details a case in which fatal coma developed after a period in which the diet contained 100 gm. of butter per diem.

An interesting experiment by Satta indicates that the grade and character of acidosis do not vary whether the patient burns his own or ingested fat, since two subjects, one on a meat-fat diet, the other fasting, began to excrete beta-oxybutyric acid on the first and second days, the total excretion averaging 7.89 gm and 7.85 gm. daily over the three or four days of observation. Yet in the fasting subject the acidosis increased rather more rapidly. Allard found that the introduction of fast days in the course of diabetes caused a marked reduction of acetone lasting several days, an observation that shows that acetone compounds are rapidly produced from ingested food.

While the above clinical experiments strongly impressed the belief that fats are the chief source of acetone compounds, this opinion has long lacked a needed confirmation from the inability to trace the steps through which fats become converted into acetone compounds. This deficiency has been largely met by Knoop, who has shown that fatty acids are attacked by oxidation at the beta-carbon atom, and that, according to this rule, certain fatty acids are readily oxidized in the body to the acetone series. Knoop executed the plan of attaching the benzol ring to various fatty acids to facilitate their identification and then determining their fate in fasting dogs. In this way he was able to arrest the oxidation at certain points so as to determine the steps in the process.

If the fatty acid attached as a side chain to the benzol ring contains only two carbon atoms, as phenyl acetic acid, $C_6H_5CH_2COOH$, these can not be split off and such compounds pass through the body unaltered. If the chain contains three or four carbon atoms these can be split off, but only in groups of two. Thus:

Cinnamic acid, $C_6H_5CH=CHCOOH$, yielded C_6H_5COOH .

Phenyl butyric acid $C_6H_5CH_2CH_2CH_2COOH$, yielded
 $C_6H_5CH_2COOH$.

Phenyl valeric acid, $C_6H_5CH_2CH_2CH_2CH_2COOH$, yielded
 C_6H_5COOH .

On the other hand, phenylalanin, $C_6H_5CH_2CHNHCOOH$, was completely burned, the benzol ring being split up. Phenyl lactic acid,

$C_6H_5CH_2CHOHCOOH$, and some other benzene compounds were also split up, but the conditions determining the combustion of the benzol ring have not been defined.

Knopp's beta-carbon oxidation theory has revealed for the first time a definite rule governing the oxidation of fatty acids, and if his deductions are valid then it should become possible to predict from their chemical constitution which acids will prove ketogenic. In order to yield beta-oxybutyric acid a normal fatty acid must contain at least four carbon atoms. It must contain an even number of carbon atoms in order to follow the rule of beta-carbon oxidation, with splitting off of the carbon atoms in groups of two. Thus normal valeric acid ($CH_3CH_2CH_2CH_2COOH$) does not yield beta-oxybutyric acid, while caproic acid does ($CH_3CH_2CH_2CH_2CH_2COOH$) (Schwarz).

The theory has already been tested to some extent. Embden, by perfusing excised organs with aerated blood, has found that the liver is the sole organ producing acetone under these conditions. Perfusion through lung, muscle or kidney failed to give acetone (Embden, Kalberlah). He, therefore, located in the liver the chief function of acetone production in the body.

Embden, Salomon and Schmidt, perfusing through the excised liver aerated blood containing various fatty acids, obtained results which show that the rule of beta-carbon oxidation does not apply in the case of branched fatty acids or amino acids. Thus leucin and isovaleric acid gave increased acetone while aminovaleric acid and isobutylic acid did not.

Yet isovaleric acid, $\begin{array}{c} CH_3CH_3 \\ | \\ CH \\ | \\ CH_2 \\ | \\ COOH \end{array}$ can not give beta-oxybutyric acid by oxidation

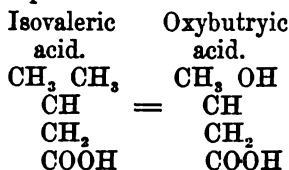
at the beta-carbon atom. Baer and Blum obtained similar results, conflicting with this theory. Administering fatty acids to diabetics, they found that isovaleric acid, leucin, tyrosin, and phenyl-

alanin, were ketogenic, while normal valeric acid, $\begin{array}{c} CH_3 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array}$ and isobutyric

acid, $\begin{array}{c} CH_3CH_3 \\ | \\ CH \\ | \\ COOH \end{array}$ were not.

Borchardt and Lange seem to have thrown needed light on the subject by pointing out that three rules govern the formation of beta-oxybutyric acid from fatty and amino acids:

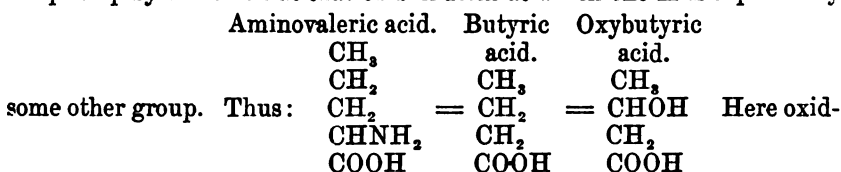
1. Branched fatty acids yield beta-oxybutyric acid through the replacement of the methyl group (CH_3) by hydroxyl. Example:



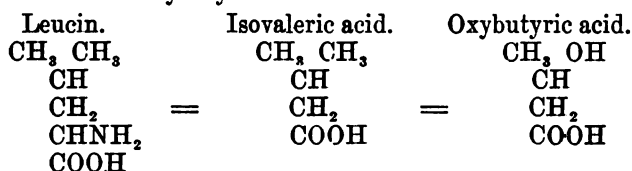
Schwarz, Emden, and Baer and Blum, all

found isovaleric acid ketogenic.

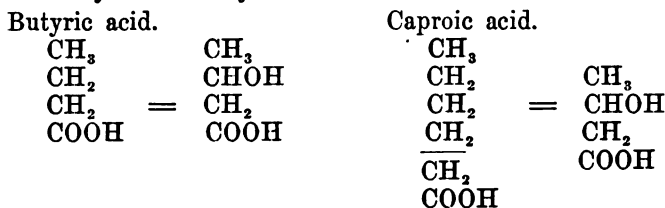
2. Derivatives of fatty acids and proteins, including amino-acids, may be split up by oxidation at that carbon atom at which one H is replaced by



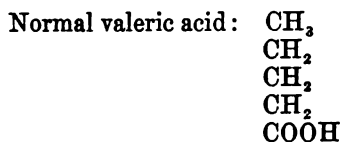
ation occurs at the alpha-carbon atom, and since amino-acids of this type constitute the bulk of most proteins, this rule may explain the possible ketoplastic influence of many proteins. Thus leucin passes through isovaleric acid to beta-oxybutyric acid.



3. Normal straight-chain fatty acids follow Knoop's rule of beta-carbon oxidation, and they yield beta-oxybutyric acid only when they contain at least four and an even number of carbon atoms. Butyric and caproic acids yield beta-oxybutyric acid.



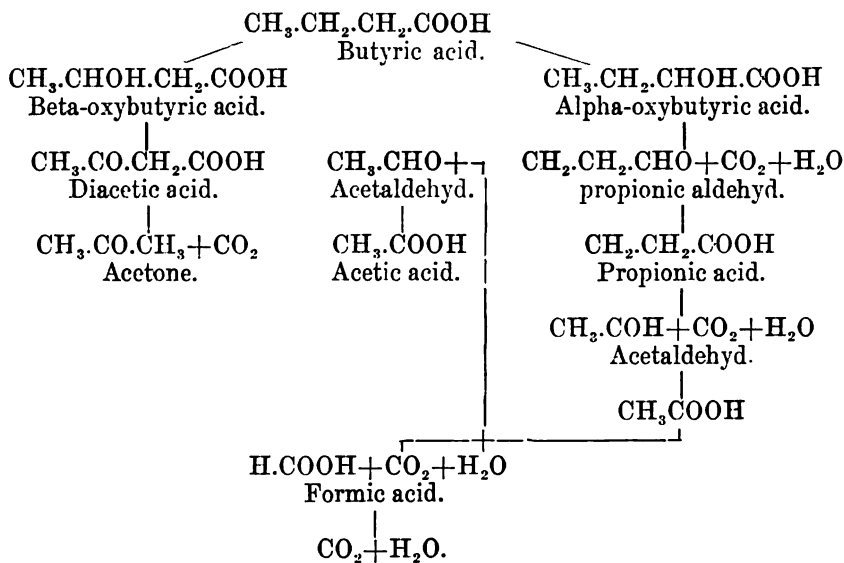
Normal valeric acid, however, is not ketogenic, oxidizing at the beta-carbon atom.



How far these rules may explain the behavior of other fatty acids which have not been directly tested, and especially their application to more complex fat and protein derivatives, remains for future studies to determine. So far as I can learn these rules cover the observed facts.

Many of the obscurities in the working out of Knoop's theory are missing in the direct demonstration by Dakin of the steps of oxidation of butyric acid in the test-tube under the influence of peroxid of hydrogen. The convincing quality of Dakin's work consists in the direct isolation from the products of oxidation of butyric acid of a number of substances which when arranged in their necessary series reveal the steps which the process has taken. Assuming that the higher fatty acids when breaking down reach the stage of butyric acid by the rule of beta-carbon oxidation, Dakin isolated from the further oxidation of butyric acid the following substances: aceto-acetic acid, acetone, propionic aldehyd, acetaldehyd, acetic acid, formic acid and carbon dioxid. From these data the following series of changes has been reconstructed by Dakin.

DERIVATION OF ACETONE FROM BUTYRIC ACID.—DAKIN.



According to this scheme butyric acid under these conditions suffers oxidation at both alpha- and beta-carbon atoms, in the former case yielding acetone compounds, in the latter case passing through a longer series of steps, possibly including lactic acid, but eventually reaching complete oxidation with avoidance of the acetone stage. This conception of the breaking up of fatty acids applying equally to the amino-acids, opens up

several lines of speculation. It would appear to be a matter of considerable consequence whether oxidation occurs at the alpha- or the beta-carbon atom, since in the former case the process becomes arrested at the acetone stage, while in the latter case, passing, as Dakin believes, through lactic acid, the process is carried to completion. Much importance may therefore attach to the decision whether oxidation shall occur at the less favorable beta-carbon atom or in the more propitious alpha position.

The significance of Dakin's work must eventually depend on the validity of reactions in the test-tube for processes occurring in the body. It is pointed out that similar products of protein decomposition result from the action of bacteria and yeasts. Yet hydrogen peroxid does not act in the body, and even in the test-tube its behavior is easily influenced. Shaffer has shown that uric acid is rapidly broken up in the test-tube by hydrogen peroxid, but on the addition of a little of the enzyme katalase, hydrogen peroxid is promptly split up and the uric acid remains unaltered.

At present we must be content to learn that such a series of changes as Dakin constructs is a possibility, while leaving to the future to decide its bearing on vital processes.

RELATION TO PROTEINS.

The possibility of obtaining acetone from proteins in the test-tube seemed to have been proved by Cotton by digesting fibrin and casein with peroxid of hydrogen, and by Blumenthal and Neuberg by heating gelatin with ferric salts and peroxid of hydrogen, while Orgler in the same manner obtained acetone from crystalline egg albumin.

The clinical observations pointing to the derivation of acetone compounds from proteins relate chiefly to diabetic acidosis. The well-known fact that diabetics respond to meat-fat diet with increased acetonuria was long accepted as proof of the protein origin of acetone, and it still remains a question whether, under these conditions, much of the acetone in diabetes is not the result of protein metabolism. The acetonuria following withdrawal of carbohydrates was also interpreted in the same direction.

On the other hand, cases of diabetes were observed, two of which were carefully reported by Weintraud and by Magnus-Levy, in which with nitrogen equilibrium, large amounts of acetone compounds were excreted over long periods. Further, Hirschfeld, Palma, and Waldvogel showed that there was usually no parallel between nitrogen and acetone excretion, and in three cases Hirschfeld found less acetone with rich

Not all radicles of the protein molecule seem to produce acetone, at least in the animal body. In fact, it is clear that proteins, like fats, contain antagonistic groups as regards acetone formation. Some indication of the nature of these antagonistic radicles may be obtained from an inspection of the various protein derivatives whose relation to acetone formation has been tested.

KETOGENIC RELATIONS OF PROTEIN DERIVATIVES.

KETOGENIC.

Leucin.....	Borchardt, Lange.
Arginin.....	Borchardt, Lange.
Aminovaleric acid.....	Borchardt, Lange.
Isovaleric acid.....	Baer, Blum.
Beta-aminobutyric acid.....	Sternberg.
Nutrose, casein (in diabetes).....	Lüthje.
Protamin.....	Borchardt.
Histon.....	Borchardt.
Egg albumin.....	Borchardt.
Thyroid.....	Waldvogel.
Sodium butyrate.....	Schwarz.
Sodium acetate.....	Satta.
Butyric acid (in diabetes).....	Loeb, Mohr.
Oxybutyric acid (in diabetes).....	Loeb, Mohr.

ANTI-KETOGENIC.

Alanin.....	Satta, Borchardt, Lange.
Lactic acid.....	Satta, Borchardt, Lange.
Aspargin.....	Satta, Borchardt, Lange.
Glutaric acid.....	Baer, Blum.
Glycerin.....	Hirschfeld.
Sugars, saccharose, glucose, maltose, mannite.....	Hirschfeld.
Alcohol.....	Neuberg.

NEGATIVE.

Glycocoll, glutaminic acid, glycolic acid, acetic acid.....	Borchardt, Lange, Baer, Blum.
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In the above lists it is evident that the antiketogenic substances belong in general in the carbohydrate class.

Among the ketogenic substances are fatty acid derivatives, certain monamino-acids, and more complex protein compounds. The behavior of the amino-acids and the complex protein compounds presents some obscure features. While pure leucin and aminovaleric acid are ketogenic, the study of the influence of protein foods containing these acids shows that such foods are relatively antiketogenic, and in direct proportion to their content of monamino-acids. Borchardt finds that protamin, histon, egg albumin, pancreas and casein contain monamino-acids increasing in the order named, and that in healthy subjects showing acetonuria from

carbohydrate-free diet protamin markedly increases acetone, while casein reduces it. Likewise Rosenthal finds that meat, casein, egg albumin, and thymus, added to rich protein diet, all increase acetone, but the increase is least with casein which contains the highest proportion of monamino-acids. It is, therefore, necessary to suppose that proteins contain antiketogenic as well as ketogenic radicles.

Certain antiketogenic radicles of proteins are not difficult to trace, since they appear in the carbohydrate reactions which have long been recognized and are divided among the hexoses and pentoses, etc. In egg albumin Müller and Seeman estimate that 20 per cent. of the carbon is in the form of radicles with carbohydrate tendencies.

The position of the monamino-acids in this respect is uncertain. The fact that in pure form leucin is ketogenic shows that these acids act as sources of acetone. Yet the relatively antiketogenic influence of proteins rich in monamino-acids indicates that in their natural relations these acids tend to act more like carbohydrates and reduce acetone. Possibly in different conditions they may turn in either direction.

The following table from Rosenthal exhibits these general relations, showing that the higher the glycogen-producing quality and the content of monamino-acids, the less is the production of acetone.

KETOGENIC RELATIONS OF PROTEINS ON RICH PROTEIN DIET—(Rosenthal).

Protein substance.	Glycogen forming capacity.	Monamino-acid N Kossel-Kutscher.	Acetone excretion.
Meat	Abundant.	Rich.	899 mg.
Casein	3.00 %	53.70 %	1113 mg.
Egg albumin	1.97 %	50.40 %	1498 mg.
Thymus	0.284 %	26.88 %	1863 mg.

In diabetes the influence of these proteins will depend on the extent to which carbohydrate combustion is lost. Thus Luthje found that calf thymus, poor in monamino-acids, reduced diabetic acidosis, while casein, rich in these acids, usually increased the acidosis. Here the monamino-acids appear to be ketoplastic. There is, therefore, evidence indicating that leucin acts differently in diabetes and in health, and that its behavior when given in pure form may vary from that observed when it is administered in the whole protein.

The work in this field reaches a practical bearing in the choice of protein foods which may inhibit acidosis. It is well known that the various proteins, especially those of vegetable origin, differ enormously in their content of monamino-acids. Quantitative analytic methods for this work are not yet fully available, but the contributions of Fischer, Müller, Hausmann, Kossel, Abderhalden, Osborne and Barker reveal from this standpoint a new outlook for rational dietetics. It is known that the

common meats do not differ greatly in monamino-acids, which constitute 60 to 66 per cent. of these proteids, but in thymus the proportion is below 50 per cent.

The demonstration that proteins contain abundance of acetone-formers does not assure that they figure prominently as a source of acetone in disease. Their relation to the different forms of acidosis has to be determined by direct observation, and the present tendency is to attribute to proteins a very subordinate rôle as sources of acetone; but the recent studies in protein chemistry have reopened the whole question, which seemed at one time practically settled in favor of the fats.

RELATION OF ACETONE COMPOUNDS TO AMMONIA.

According to the theory of acid intoxication, increased excretion of acetone compounds should be accompanied by increased excretion of ammonia. In the majority of cases of acidosis, especially in diabetes, this relation holds and the ammonia excretion has come to be regarded as the measure of acidosis. Yet the exceptions to the rule are so numerous and striking as to raise grave doubts regarding a necessary relation between ammonia and acetone compounds and acidosis in general.

Limbeck has shown that excessive doses of alkalis do not remove all ammonia from the urine, which must have other functions than the neutralization of acids. In many cases of diabetes and other diseases associated with acidosis, ammonia excretion is considerable while acetone compounds are scanty or absent. In acidosis the ammonia bears relation not only to the organic acid excretion, but also to the total nitrogen excreted, so that in any attempt to estimate acidosis from ammonia it becomes necessary to consider not only the absolute quantity but also the percentage of nitrogen excreted as ammonia.

Satta has considered in detail the relation between ammonia and acetone compounds in healthy subjects on carbohydrate-free diet. On feeding glycerin or fats under such conditions the ammonia-acetone compound ratio varied excessively, the acetone compounds rising far out of proportion to the ammonia and the ratio falling. An influence of excessive inorganic acids in the food was eliminated, since these remained constant. The increase of ammonia occurred on the first day of the experiment, although the blood contains alkali capable of neutralizing 80 gm. of beta-oxybutyric acid. In diabetes, 20 gm. of sodium bicarbonate failed greatly to influence the ammonia or the acetone compounds, although the amount of alkali given was sufficient to neutralize more than the total quantity of acetone compounds.

AMMONIA: ACETONE COMPOUNDS RATIO. SATTA.

Urine, c.c.	S. G.	N.	NH ³ .	Acetone cmpds. as oxybut. acid.	NH ³ :ace- tone cmpds.	Diet.
2290	1.010	14.4	0.856	0.95	.90:1	Meat.
1930	1.010	10.8	1.42	1.91	.74:1	Meat.
1660	1.015	10.9	2.51	8.73	.28:1	Meat.
2150	1.011	10.7	3.40	20.0	.17:1	Meat.

In a second case of diabetes 40 gm. of sodium bicarbonate reduced the acetone compounds to a trace, although 2.64 gm. of ammonia (= 13.2 gm. beta-oxybutyric acid) were excreted.

Allard also has emphasized the very loose relation existing between NH₃ and beta-oxybutyric acid in diabetes when the hourly variations are followed, observing that on meat-fat diet the ammonia does not follow the variations in acetone compounds, while on hunger days the ammonia rises, although the acetone compounds diminish. The influence of the alkalies of the food may in part explain these discrepancies.

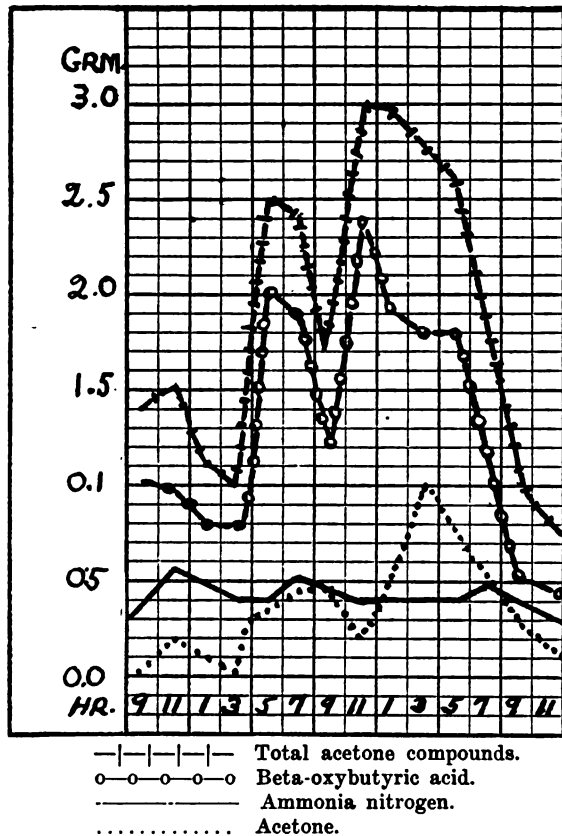
HOURLY EXCRETION OF ACETONE COMPOUNDS AND AMMONIA IN SEVERE DIABETES, ON MEAT-FAT DIET. ALLARD.

Hence Satta concludes that the ammonia formation in acidosis is largely the result of a specific disorder of nitrogenous metabolism and is not merely concerned with neutralization of acids. While regarding the ammonia as an index of the disturbed nitrogenous metabolism, he would consider the acetone compounds as a measure chiefly or exclusively of disturbed fat metabolism. An uncertain element in these deductions is the possible presence of acids not considered in the computations. The ammonia excretion in other forms of acidosis bears on this question and will be considered later. Here it may only be suggested that the estimation of acidosis by ammonia excretion is an indirect and often very unreliable measure of acidosis and is an especially uncertain index of any particular type of acidosis.

INTESTINAL ORIGIN OF ACETONURIA.

The whole subject of the exact sources of acetone would lose much interest if it should transpire that any considerable degree of acetonuria is of intestinal origin. This was, indeed, one of the earliest interpretations of acetonuria, being introduced by Lorenz in 1891, and being once widely accepted as the probable explanation of many toxic symptoms accompanying gastrointestinal disorders. Against the intestinal origin of the acetone compounds in gastrointestinal diseases stood the prominent fact that the patients were usually imperfectly nourished, and Waldvogel has clearly emphasized the importance of starvation and burning of body fats in these cases. Moreover, the direct examination of the

stools failed as a rule to show a sufficient depot for the urinary acetone. Yet Müller reports the presence in Cetti's stools of 1.21, 0.57, 1.14 gm. of fat, one-half neutral fats and cholesterin, the other half free fatty acids and soaps. In Nebelthau's case, with 24 to 43 mg. acetone in the daily urine, the feces (108 gm. dry) contained 0.103 gm. of acetone. In addition to the element of starvation, Satta considers the influence of absorbed toxic substances to be of considerable importance in this form of acetonuria.



The prompt antiketogenic action of carbohydrates has been considered as favoring the intestinal origin of acetone, but this rapid influence is undoubtedly connected with the easy absorption of many carbohydrates. Likewise, the failure of sugars, when introduced by rectum, to influence acidosis has been set aside by Satta, who finds that sugars administered by rectum are antiketogenic if given in considerable doses and

properly absorbed. Definitely against the theory of intestinal origin of acetone is the fact that cathartics and intestinal antiseptics, such as benzol and salol, frequently fail to influence acidosis or may even increase it. In diabetic coma Magnus-Levy and Geelmuyden found less acetone proportionally in the intestine than in the organs. In diabetes, as in cyclic vomiting of children, the acetone in the stools and occasionally in the vomitus is most probably an evidence of excretion by these channels.

If acetone is derived from decomposition of protein food, then it should run parallel with sulphur excretion. Yet no such parallel but rather the opposite relations appears in the observations of Cetti.

In starvation acetone increases steadily during the first week while the intestinal contents diminish: the intestinal canal does not contain enough protein in starvation to yield 10 gm. beta-oxybutyric acid, the amount excreted by Cetti, while an origin from intestinal fat is excluded by the scanty content of the stools in fats.

Finally, the enormous amounts of acetone compounds in diabetes on fasting days seem to exclude an intestinal origin and point to processes of internal metabolism as the sole source of the compounds. Hence, with the increasing knowledge of the factors influencing acidosis, the theory of intestinal absorption has steadily lost ground, and is to-day quite generally abandoned.

TOXICITY OF ACETONE COMPOUNDS.

The foregoing review of the sources and relations of the acetone compounds warrants the conclusion (now generally accepted) that these substances result chiefly from the incomplete and probably abnormal burning of fats induced by the absence of carbohydrates. The sugar-hungry cell—to use the apt expression of Lusk—turns to the fats for energy and secures it at the expense of a disordered and incomplete oxidation. In diabetes probably a considerable proportion of the acetone compounds come from the deamidized fatty radicles of the protein molecule, but in other conditions important protein sources of acetone have not been demonstrated.

Having traced the origin of the acetone compounds, the important question of their physiologic action remains to be considered. Are there reasons for supposing that the acetone compounds are directly toxic and can the symptoms associated with acetonuria be referred to the direct toxicity of these substances?

It was von Jaksch's opinion in 1885 that acetonemia was the direct cause of a great variety of symptoms associated with acetonuria, but numerous studies of the toxicity of acetone before that time, especially

those of Kussmaul, and many subsequent reports have succeeded in showing that acetone acts much like alcohol or chloroform, occupying an intermediate position between these two narcotics; that it is incapable of inducing the nervous symptoms of fever, gastroenteritis, carcinoma, psychoses, or of other conditions marked by acetonuria; that it is quite incapable of producing the symptoms of diabetic coma, and that the doses required to produce symptoms in man or experimental animals exceed the amounts excreted even in diabetics. Twenty grams, or 0.08 gm. per kilo (Röhrig), produce only transient somnolence in man, while 0.2 gm. per kilo causes only mild intoxication. In dogs Schwarz caused seven intoxication with 15.3 gm. by mouth. Dreschfeld observed no symptoms after taking 20 gm. of acetone, or after giving moderate doses to diabetics, but five or six injections of 10 minims of acetone in rabbits produced coma, slow breathing, convulsions and albuminuria. He concluded that acetone is non-toxic to healthy animals, but in disease when excretion was diminished he thought it might be responsible for serious symptoms, even those of diabetic coma. Yet the symptoms he produced were not those of diabetic coma, while the doses employed were far larger than occur in diabetes.

I have given acetone to healthy rabbits subcutaneously, 6 gm. daily, for three to five days, causing dulness and drowsiness, with slight albuminuria and acetonuria. After twelve days death occurred in one instance with emaciation and drowsiness but without coma and without fatty changes in the liver or kidneys. The urine was always alkaline, with slight acetone, and a very heavy precipitate of carbonates.

With the idea that acetone might prove more toxic in animals with fatty liver, I anesthetized a white rabbit very deeply with chloroform for thirty-five minutes, and on the following day three times for periods amounting to forty-five minutes in all. The animal recovered with great difficulty. On the following day 2 gm. of acetone at one injection failed to produce any symptoms except marked acetonuria, and 4 mg. daily on successive days were also without effect.

Negative results from the injection of acetone compounds in healthy subjects or even in diseased animals may be inadequate to decide its action when spontaneously arising in the course of the disease, but the futility of attempting to refer diabetic coma to this agent has been generally admitted. Its possible relation to milder symptoms in various diseases must be determined by future studies. At present its behavior in disease, the lack of relation to the severity of the process, its absence in many cases which show characteristic symptoms, and abundance in other cases free from signs of intoxication, do not favor the belief that it is a prominent factor in any symptom-complex in man.

With aceto-acetic acid very conflicting results have been reported. Von Frerichs, von Jaksch, Albertoni, and Dreschfeld found it non-toxic in man, even in excessive doses.

The optically inactive beta-oxybutyric acid has been tested by a large number of observers, especially by Sternberg in large doses, 10 to 12 gm., in healthy men and animals, and in diabetics, with nearly uniform failure to produce symptoms of intoxication, although the acid is a decided local caustic. With the optically active acid from diabetic urine Minkowski and Schwarz failed to detect toxic symptoms in normal or diabetic dogs. Waldvogel was able to introduce 2.2 gm. of the acid intravenously in the phloridzin-poisoned rabbits without symptoms, although much smaller doses twice proved fatal. Subcutaneously 1.1 gm. caused hemorrhagic nephritis and death in two days. Herter, by slowly infusing into the femoral vein of monkeys large amounts of a 4 to 5 per cent. solution of beta-oxybutyric acid in normal salt, produced narcosis, but he was uncertain as to the exact significance of this experiment.

More recently Wilbur infused rabbits at the rate of 4 c.c. per minute with N/10 sulphuric acid, acetic acid 2 per cent., lactic acid 5 per cent., and beta-oxybutyric acid 5 per cent., producing with all characteristic symptoms of fatal acid poisoning. The fatal dose with each acid was uniform, the sulphuric acid being five times as active as beta-oxybutyric acid, of which 0.03152 c.c. of 5 per cent. solution per gram of body weight was fatal. Of sodium beta-oxybutyrate 0.116 c.c. of 5 per cent. solution per gram of body weight was fatal. After giving 75 c.c. of sodium salt, 24 c.c. of 5 per cent. free beta-oxybutyric acid was given, causing convulsions and death. Even 5 c.c. of the acid after the usual dose of sodium salt was very toxic. Wilbur, therefore, concludes that the salts of beta-oxybutyric acid are toxic, and that in diabetes the action of this acid is not all due to its acid properties.

Reference may again be made to the experiments of Harley, who produced in dogs coma like that of diabetes by ligating the ureters and injecting 8 to 12 gm. of glucose per kilo. The glucose disappeared from the blood in four to six hours and at the same time coma developed and deepened as the glucose diminished, while the blood alkalescence and carbon dioxide decreased and lactic acid increased from 0.09 per cent. to 0.134 per cent. The symptoms were referred to acid products of glucose.

Sternberg produced in dogs a condition resembling diabetic coma by infusions of aminobutyric acid, but this substance is probably not a natural product of intermediary metabolism.

Familiar objections meet all these attempts to duplicate diabetic coma experimentally. It is generally recognized that the rapid introduction of

large quantities of acids into the blood is too crude and the factors involved are too complex to render the experiment convincing. Observations on the occurrence of beta-oxybutyric acid in disease have far more decisive value, and when 40 gm. of this acid may be excreted in one day in a case of syringomyelia on a meat diet, without symptoms of intoxication, it is difficult to maintain that this acid exerts any immediate toxic action (Gerhardt, Schlesinger).

As an indirect effect of the long-continued excretion of acetone compounds must be considered the nephritis which most observers describe among the results of poisoning by acetone and oxybutyric acid. Ebstein and Kulz called attention to the appearance of very numerous small hyaline and granular casts in diabetic coma, and Waldvogel and others regard the appearance of such casts as a sign of excessive excretion of acids, since they have been found to disappear on feeding carbohydrates. In the experiments with hydrochloric acid, oxybutyric acid and acetone, albuminuria, casts and nephritis are nearly always noted. In clinical experience also long-continued acidosis is often followed by nephritis, especially in the toxemia of pregnancy, while nephritis is nearly constant in the terminal stages of prolonged diabetes. Many considerations of this character suggest that the acetone compounds, in addition to their acid action, are indirectly toxic through injury to the renal cells and the production of a progressive nephritis.

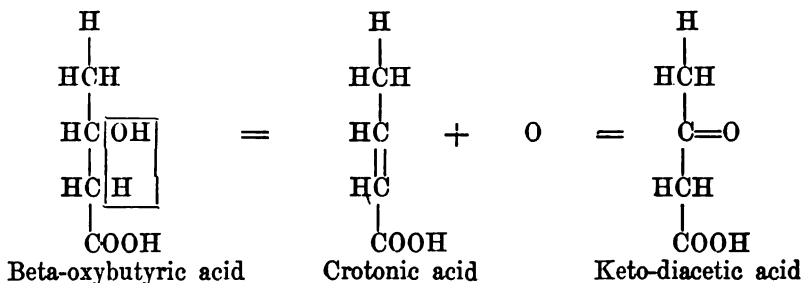
RELATION OF ACETONE COMPOUNDS TO OXIDATION.

A great mass of evidence, clinical, pathologic and chemical, as well as prevailing opinion, points to deficient oxidation as concerned in the formation of acetone compounds. Clinically the diseases accompanied by acidosis are marked by excessive consumption or by deficient supply of oxygen, often by striking disturbance of respiration and by deficient general vitality, but it must be admitted that such points of view offer very uncertain data. The main basis of the theory of suboxidation is found in the general pathologic relations of fatty degeneration, which have been fully stated by Klebs and many others, and the evidence from these sources is rather comprehensive and consistent, but the further one pursues this theory in the analysis of familiar pathologic processes the less adequate it appears. It is true that in a moribund patient oxidation is less active than in health, but the statement of this fact does not add much to the conception of lethal diseases. That oxidation in many conditions does not proceed with normal vigor is undeniable, but that any definite pathologic process consists essentially in a low grade of oxidation appears still to require demonstration. As Speck concludes from a review of the

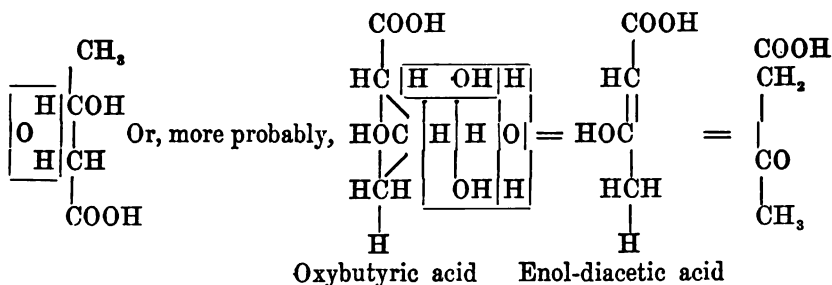
chemical reactions concerned in metabolism, life does not involve simply oxidative processes.

According to Nasse, the metabolism of carbohydrates activates oxygen in the body and thereby furthers the oxidation of fatty acids to their normal end products. Yet it is difficult to see how the katabolism of glucose can liberate any free oxygen. The study of intoxication acidoses by Winterstein and Boeri led them to conclude that the assimilation of oxygen was interfered with by the poisons. Yet Lusk has shown that in phosphorus poisoning the interchange of oxygen by respiration is equal to that in health. After extirpation of the liver urea fails to be formed, but whether the defect is one of oxidation, hydrolysis, or dehydration, is not known, while the absence of specific ferments normally supplied by the liver explains the condition much more adequately than does the absence of any one chemical reaction. In diabetes there is a failure of certain processes in which oxidation is concerned, as the burning of fatty acids. Yet we do not speak of diabetes as a form of suboxidation but emphasize rather the absence of specific ferments which call into play other chemical processes as well as oxidation. Hence the ready resort to the phrase "suboxidation" in the theory of diseases of metabolism is inaccurate and misleading.

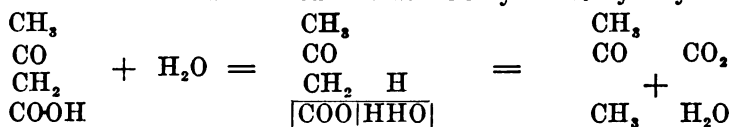
The extent to which oxidation figures in the physiologic chemistry of acidosis may be determined by the character of the reactions involved in the formation and katabolism of the acetone compounds. Here it is evident that oxidation, dehydration and hydrolysis are variously combined. In the formation of diacetic from oxybutyric acid one may assume that either the enol or the ketol form of diacetic acid is produced. In either case both dehydration and oxidation are combined.



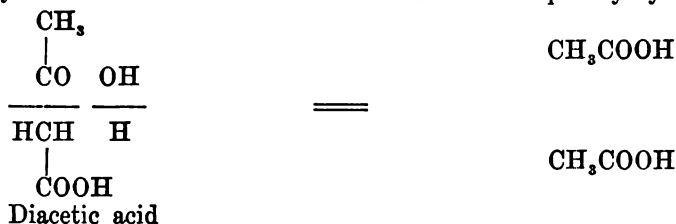
With keto-diacetic acid dehydration of beta-oxybutyric and oxidation through crotonic acid are necessary. In the case of enol-diacetic acid dehydration and oxidation with formation of three molecules of water are involved.



Diacetic acid is transformed into acetone by direct hydrolysis.



It is possible, however, that diacetic acid is directly broken up by hydrolysis into two molecules of acetic acid and subsequently hydrolyzed.



Hence oxidation and hydrolysis are both involved even in the simpler final steps of formation of acetone compounds. The possibility of the synthetic origin of beta-oxybutyric acid has been discussed by several writers without definite conclusions. When one turns to the field of the earlier cleavage processes in the formation of lower fatty acids from fats and proteins the relation of oxidation and hydrolysis becomes even less certain. From the general chemical standpoint Folin expresses the opinion that the relative importance of oxidation and hydrolysis in the production of beta-oxybutyric acid is still undetermined.

Dakin's experiments in the treatment of fatty acids with peroxid of hydrogen compel the conclusion that one essential factor in his conversion of these acids into acetone compounds, etc., is oxidation, but hydrolysis is also concerned.

Confirmatory evidence that certain definite oxidative processes in the body are deficient in some diseases accompanied by acidosis is furnished by the studies of Richards and Howland on cyclic vomiting of children, and of Richards and Wallace on the influence of cyanid on metabolism, which show that in this form of acidosis neutral sulphur is notably in-

creased. In the case of the sulphur, at least, there can be no doubt that a process of oxidation is involved.

It is impossible here to enter further into the complex ramifications of the subject of the general significance of oxidative processes in the body. It must suffice to point out that in acidosis, while deficient oxidation is an essential element, it is only one of several factors involved.

PHYSIOLOGY OF LACTIC ACID.

CH_3
Although lactic acid CHOH is chemically a fatty acid, it is much
 COOH

more closely connected with the carbohydrates and proteins than with the fats, from which, however, Dakin obtained it by warming higher fatty acids with hydrogen peroxid. It is produced chiefly in the muscles, probably from carbohydrates (glycogen, glucose), and is either burned in the muscles or carried to the liver for combustion. In phloridzinized dogs 70 per cent. of ingested lactic acid may be synthesized to glucose. Lactic acid may also be derived from glucose by the action of a ferment contained in animal tissue (Stocklasa). Embden obtained much lactic acid by perfusing blood through a liver containing glycogen, but less when the liver was free from glycogen. Hence, Mandel and Lusk point out that a series of transformations exists—lactic acid, glucose, glycogen, glucose, lactic acid.

In herbivora lactic acid readily appears in the blood and urine in large amounts. After extirpation of the liver in geese, Minkowski found 3.5 gm. in the urine of the eighteen hours during which the animal survived the operation; it was combined with ammonia, with which it replaced the bulk of uric acid. Here it resulted from the loss of synthetic function of the liver. In this case the lactic acid must have been derived from proteins since it was proportional to the nitrogen excretion and was not increased by giving carbohydrates. Protein derivatives, like alanin and leucin, are readily convertible into lactic acid by hydrolysis.

In rabbits with strychnin tetanus it is abundant in the urine, greatly reduces the carbon dioxid content and titratable alkali of the blood, and leads to an extreme acidosis (Araki). Here it is a product of excessive muscular activity, while the deficient oxidation which results from the great dyspnea of strychnin poisoning permits it to escape oxidation and appear in the urine. Similar results appear in man after epileptic convulsions and in soldiers after exhausting marches (Inouye, Saiki). Yet carnivora burn lactic acid much more actively than herbivora and also enjoy the sources of ammonia as a protective mechanism, and it is doubtful if any dangerous grades of acidosis from lactic acid occur in man.

Kraus has pointed out that those conditions in which beta-oxybutyric acid is prominent fail to show much lactic acid. This fact is partly owing to the difference in the sources of lactic acid and of the acetone compounds. Lactic acid also has a pronounced antiketogenic influence. There is, therefore, a definite chemical, physiologic, and clinical antagonism between these two forms of acidosis, and this principle I believe may furnish a basis for the classification of diseases associated with acidosis.

The mechanism concerned in the production of lactic acid and its appearance in the blood and urine is probably a special form of defective oxidation. According to Hoppe-Seyler all tissues containing glycogen or glucose produce lactic acid when imperfectly supplied with oxygen. Araki in a series of experiments has endeavored to establish this principle in the production of lactic acid. By slow partial asphyxia, poisoning by carbon monoxid, curare, strychnin, morphin, amyl nitrite, phosphorus, and arsenic, he has found moderate or large amounts of lactic acid in the blood and urine, often with glycosuria, and has traced the influence of deficient supply of oxygen in these conditions.

Whether quantitative deficiency or the inability of the cells to appropriate the oxygen is the essential factor is uncertain.

In hydrocyanic acid poisoning oxygen is in excess, but the cells can not appropriate it (Geppert). After extirpation of the liver it would appear that special ferments or other factors must be deficient.

In phosphorus poisoning the classical type of defective oxidation is believed to exist. Lusk has shown that in phosphorus poisoning in dogs the interchange of oxygen by respiration is normal, but it is still possible to suppose that while the respiratory currents are normal part of the oxygen is diverted from normal oxidative functions to an excess of intermediary processes of metabolism so that some oxidative processes are not carried to completion.

Although in dogs with Eck fistulas the ammonia seems to be an immediate result of the loss of urea-forming function of the liver, in phosphorus poisoning the ammonia appears to be somewhat dependent on the presence of lactic acid. Ray, McDermott and Lusk, by administering phloridzin to phosphorus-poisoned dogs, caused both the ammonia excess and the lactic acid to disappear simultaneously. Now the effect of the phloridzin is merely to prevent the formation of lactic acid, in the absence of which acid there would be no need of ammonia. Hence, the disappearance of ammonia after phloridzin poisoning would seem to indicate that the ammonia was not the direct result of injury to the liver, but was only a protective agent against the lactic acid. Yet it

appears possible that phloridzin may also inhibit desamidation and thus diminish the ammonia. In either case the lactic acid and ammonia signify defective action of the liver, in the first instance from the lack of urea formation, in the second from failure to burn lactic acid, both of which functions the normal liver accomplishes.

Lactic acid appears to exert a controlling influence over fatty infiltration of the tissues. Rosenfeld finds that there is a physiologic antagonism between glycogen and fat deposit in the liver. When this organ has abundance of glycogen at command visible fat is not present, but when glycogen is lacking it must have fat for the supply of heat, and drawing it from the fat depots, visible fat appears in the liver cells.

In fasting phloridzinized dogs acidosis begins only after the liver has consumed its glycogen and has begun to burn fats and proteins.

When lactic acid appears in the urine it is a sign that glycogen is not completely burned. Under such conditions fatty changes in the liver are very constant. According to Lusk the "sugar-hungry" cell attracts fat in larger quantity than can be burned, so that a deposit of fat occurs in the cell. Hoppe-Seyler and Araki find that diminished oxygen supply results in the appearance of lactic acid. For this reason, therefore, lack of oxidation must result in fatty degeneration.

In diseases terminating in prolonged dyspnea fatty degeneration of the centers of liver lobules may or may not occur. It would be interesting to trace the relation of lactic acid in such cases. In fact, association of lactic acid in the urine with extensive fatty degeneration of the organs in many diseases deserves more thorough study. In diabetic dogs fatty liver and lactic acid in the urine are usually observed (Lusk), but in human diabetes fatty liver is rare, and Magnus-Levy believes that in this disease lactic acid occurs in negligible quantity. The reason for this difference, if it exists, may perhaps be found in the fact that dogs are less accustomed to burn carbohydrates in the food and may more readily lose all capacity to do so. Throughout the clinical forms of acidosis lactic acid and fatty degeneration are always associated.

SUMMARY.

Having sketched in some detail the form and sources of our knowledge of the substances concerned in acidosis, it remains to emphasize in summary the main results in this field.

1. While all classes of foodstuffs yield acetone compounds in the test-tube, yet in the body these compounds are derived mainly from the fat tissues and to a less extent from the food. In diabetes, however, the proteins contribute directly or indirectly to the formation of acetone

compounds. To what extent the proteins are drawn on in other conditions remains uncertain.

2. The complete combustion of fats requires the simultaneous katabolism of carbohydrates, in the absence of which there is a defective and possibly abnormal course of fat combustion lodging in the acetone compounds. In all known conditions, even in diabetes, the metabolism of carbohydrates occupies a controlling position in this form of acidosis.

3. Oxidation of straight-chain fatty acids occurs at the beta-carbon atom, so that such acids with an even number and at least four carbon atoms may yield beta-oxybutyric acid. Oxidation of pure fatty acids in the test-tube may occur at the alpha-carbon atom, with avoidance of acetone compounds, but whether this course may be followed in metabolism is uncertain. From branched fatty acids a methyl group may be replaced by hydroxyl, and in amino-acids oxidation occurs at any carbon atom holding an amino group, both of which processes may yield beta-oxybutyric acid.

Knoop's work and the ready destruction of oxybutyric acid in healthy men indicate that this acid is a normal product of metabolism.

4. The urinary ammonia is influenced by the total nitrogen excretion, by the presence of fatty acid derivatives, by lactic acid, possibly by inorganic acids, and notably by defective synthetic functions of the liver. It bears rather loose relation to the acetone compounds, and, being an indirect measure of the presence of acids, can not replace their direct estimation.

5. The grounds are still inadequate to support the view that acetone compounds, as they arise in the body, exert any notable direct toxic action.

6. Oxidation and hydrolysis are both concerned in the formation of acetone compounds.

7. Lactic acid is a product of disordered or defective katabolism chiefly of glycogen; it results also from disturbed function of the liver, and bears an important relation to fatty degeneration.

BIBLIOGRAPHY OF PART II.

- Abderhalden and others: *Ztschr. f. physiol. Chem.*, 1904-1906, xli-xlviii.
 Allard: *Arch. f. exper. Path. u. Pharmacol.*, 1907, lvii, 1.
 Albertoni and Pisenti: *Arch. f. exper. Path. u. Pharmacol.*, 1887, xxiii, 393.
 Araki: *Ztschr. f. physiol. Chem.*, 1891, xv, 335; 1892, xvi, 453; xvii, 311.
 Baer and Blum: *Arch. f. exper. Path. u. Pharmacol.*, 1906, lv, 89; lvi, 92;
Beitr. z. chem. Physiol., 1907, x, 80.
 Barker and Cohoe: *Jour. Biol. Chem.*, 1905, i, 229.
 Blumenthal and Neuberg: *Beitr. z. chem. Physiol. u. Path.* (Hofmeister's), 1902, ii, 238.
 Boeri: *Riv. clin. e terap.*, Naples, 1891, xiii.

- Borchardt: Arch. f. exper. Path. u. Pharmakol., 1905, liii, 388.
- Borchardt and Lange: Beitr. z. chem. Physiol. u. Path. (Hofmeister's), 1907, ix, 116.
- Cotton: Jour. de pharm. et de chem., x, 6, cited by Waldvogel; Chem. Centralbl., 1899, ii, 722.
- Dakin: Jour. Biol. Chem., 1908, iv, 63, 77.
- Dreschfeld: Brit. Med. Jour., 1896, ii, 358.
- Ebstein: Deutsch. Arch. f. klin. Med., 1881, xxviii, 143.
- Emlden: Centralbl. f. Physiol., 1905, xviii, 832.
- Emlden and Kalberlah: Beitr. z. chem. Physiol. u. Path. (Hofmeister's), 1906, viii, 121.
- Emlden, Salomon and Schmidt: Beitr. z. chem. Physiol. u. Path. (Hofmeister's), 1906, viii, 129.
- Folin: Tr. Assn. Am. Phys., 1907, xxii, 256.
- Fremy: Ann. f. Chem. u. Pharmakol., 1835, xv, cited by Waldvogel.
- von Frerichs: Ztschr. f. klin. Med., 1883, vi, 3.
- Geelmuyden: Ztschr. f. physiol. Chem., 1897, xxiii, 431; 1904, xli, 128.
- Geppert: Ztschr. f. klin. Med., 1889, xv, 208, 309.
- Gerhardt and Schlesinger: Arch. f. exper. Path. u. Pharmakol., 1889, xlii, 83.
- Hagenberg: Centralbl. f. Stoffwechs u. Verdauungskr., 1900, i, 33.
- Harley: Arch. f. Anat. u. Physiol., Phys. Abt., 1893 suppl., 46; Brit. Med. Jour., 1893, ii, 666.
- Hausmann: Ztschr. f. physiol. Chem., 1899, xxvii, 95; 1900, xxix, 136.
- Herrick: Jour. Am. Med. Assn., 1908, l, 861.
- Herter: Personal communication.
- Hirschfeld: Ztschr. f. klin. Med., 1895, xxviii, 176.
- Hoppe-Seylar: Festschr. f. Virchow, 71 Geburtstage, 1891, 4.
- Inouye and Saiki: Ztschr. f. physiol. Chem., 1903, xxxvii, 203.
- von Jaksch: Ueber Acetonurie und Diaceturie, Berlin, 1885; Ztschr. f. klin. Med., 1885, viii, 115.
- Jorns: Inaug. Diss., Göttingen, 1903.
- Joslin: Jour. Med. Research, 1904, xii, 433.
- Klebs: Die Allgemeine Pathologie, 1889, ii, 67, Jena, G. Fischer.
- Knoop: Beitr. z. chem. Physiol. u. Path. (Hofmeister's), 1906, vi, 150.
- Kossel: Ztschr. f. physiol. Chem., vii, viii, x, xii.
- Kraus: Ergbn. d. allg. Path. u. path. Anat., 1895, l, 593.
- Kulz: Dissertation, Marburg, 1885.
- Kussmaul: Deutsch. Arch. f. klin. Med., 1887, xiv, 1.
- von Limbeck: Ztschr. f. klin. Med., 1898, xxxiv, 419.
- Lorenz: Ztschr. f. klin. Med., 1891, xix, 19.
- Lüthje: Ztschr. f. klin. Med., 1900, xxxix, 397.
- Lusk: Proc. Soc. Exper. Biol., 1907, iv; Science of Nutrition, 247, 1907, New York, W. B. Saunders.
- Mandel and Lusk: Jour. Am. Med. Assn., 1906, xlvii, 1804.
- Magnus-Levy: Arch. f. exper. Path. u. Pharmakol., 1899, xlii, 149, 158; 1901, xlv, 389.
- Meyer: Inaug. Diss., Strassburg, 1895.
- Minkowsky: Ann. f. Chem. u. Pharmakol., 1869, cxlix, cited by Waldvogel; Arch. f. exper. Path. u. Pharmakol., 1886, xxi, 91; 1893, xxxi, 182, 214; 1898, xl, 185.
- Möhr: Samml. klin. Abhandl. (von Noorden), 1904, No. 4, Berlin, A. Hirschwald.
- Mohr and Loeb: Centralbl. f. Stoffwechs u. Verdauungskr., 1902, iv, 193.
- Müller, J.: Congr. f. inn. Med., 1898, xvi, 448.
- Müller, F.: Virchow's Arch. f. Path. Anat., 1893, cxxxi, suppl.

- Müller and Seemann: *Deutsch. med. Wchnschr.*, 1899, xxv, 209.
 Nasse: *Arch. f. d. ges. Physiol. (Pflüger's)*, 1887, xli, 378.
 Nebelthau: *Centralbl. f. inn. Med.*, 1897, xviii, 977.
 von Noorden: *Berl. klin. Wchnschr.*, 1903, 817; *Metabolism and Practical Medicine*, ii, 48, 1907, Chicago, W. T. Keener.
 Orgler: *Beitr. z. chem. Physiol. u. Path. (Hofmeister's)*, 1900, i, 583.
 Osborne and Harris: *Jour. Am. Chem. Soc.*, 1903, xxv, 323.
 Packard: *Am. Jour. Physiol.*, 1907, xviii, 113.
 Palma: *Ztschr. f. Heilk.*, 1895, xv, 463.
 Pflüger: *Arch. f. ges. Physiol.*, 1903, xcvi, 378.
 Ray, McDermott and Lusk: *Am. Jour. Physiol.*, 1899, iii, 139.
 Richards and Howland: *Arch. Pediat.*, 1907, xxiv, 401.
 Richards and Wallace: *Jour. Biol. Chem.*, 1908, iv, 179.
 Röhrig: *Inaug. Diss.*, Würzburg, 1898.
 Rosenfeld: *Deutsch. med. Wchnschr.*, 1885, p. 683; *Centralbl. f. inn. Med.*, 1895, xvi, 1233.
 Rosenthal: *Centralbl. f. inn. Med.*, 1908, 185.
 Satta: *Beitr. z. chem. Physiol. u. Path. (Hofmeister's)*, 1905, vi, 1, 388.
 Schreiber (cited by Hagenberg): *Centralbl. f. Stoffwechs.- u. Verdauungskr.*, 1900, i, 33.
 Schwarz: *Arch. f. exper. Path. u. Pharmakol.*, 1898, xl, 168; *Prag. med. Wchnschr.*, 1901, xxvi, 361, 376; *Deutsch. Arch. f. klin. Med.*, 1903, lxxvi, 247.
 Schumann-LeClereq: *Wien. klin. Wchnschr.*, 1901, 237.
 Shaffer: *Am. Jour. Physiol.*, 1905, xiv, 299.
 Speck: *Ergebn. d. Physiol.*, 1903, ii, Part 1, 1.
 Sternberg: *Virchow's Arch. f. path., Anat.*, 1898, clii, 207; *Ztschr. f. klin. Med.*, 1899, xxxviii, 65.
 Stocklassa: *Centralbl. f. Physiol.*, 1902, xvi, 712.
 Waldvogel: *Centralbl. f. inn. Med.*, 1898, 845; *Beitr. z. ges. Physiol. u. Path. (Hofmeister's)*, 1906, vii, 150; *Ztschr. f. klin. Med.*, 1899, xxxviii, 506.
 Waldvogel and Hagenberg: *Ztschr. f. klin. Med.*, 1901, xlii, 443.
 Weintraud: *Arch. f. exper. Path. u. Pharmakol.*, 1894, xxxiv, 169, 367.
 Wilbur: *Jour. Am. Med. Assn.*, 1904, xliii, 1228.
 Winterstein: *Ztschr. f. allg. Physiol.*, 1902, i, 19.

III. CLINICAL TYPES OF ACIDOSIS.

THE ACIDOSIS OF STARVATION.

One of the most significant forms of acidosis, and one that gives the clue to its origin in many diseases, occurs when the supply of food, especially of carbohydrates, is deficient. The purest examples have been shown by the professional fasters, Cetti, Breithaupt and Succi. In them acetone rapidly increased from the normal trace to a pronounced reaction a few hours after the fast began, reaching 0.5 gm. on the first day in Cetti, but only 0.054 gm. with Breithaupt, in whom the rise to 0.5 gm. required five days. The highest excretion was 0.784 gm. In many other healthy fasting men these limits were not exceeded, and often the acetone has been much lower (Müller, Senator, Hirschfeld, Waldvogel). In health most acetone (80 to 98 per cent.) (Geelmuyden) passes out by the breath, but in starvation the non-volatile forerunners of acetone can not be

readily exhaled from the lung, but are usually excreted unchanged in the urine, which thus becomes the chief channel of exit.

There are, however, striking exceptions to this rule, as in Nebelthau's case, in which only 10 per cent. of the acetone appeared in the urine. In any event, to the urinary acetone must be added 20 to 40 per cent. or more to give the total excretion in starvation.

Yet the results of the sudden withdrawal of all food in the average human being are often more pronounced. Mayer found 1.90 gm. of acetone in the urine of a fasting girl with a gastric ulcer. Diacetic acid usually appears on the first day of starvation and becomes pronounced in thirty-six hours (von Noorden). Great variations in the excretion of beta-oxybutyric acid in starvation have repeatedly been noted and may be attributed to the varying supply of carbohydrate in the body, the amount of fat in the food, the extent of the depot fat, and to individual peculiarities. In a case of hysterical vomiting Gerhard and Schlesinger found 40 gm. in one day's urine, L. Mayer 16.3 gm. in a case of gastric ulcer, and Brugsch records 9.27 to 13 gm. from the twenty-third to the thirtieth days of starvation in a professional fasting woman. These amounts are fully equal to those observed in many cases of acid diabetic coma.

It is not justifiable, however, to regard such cases as examples of simple starvation. Patients with gastric ulcer, esophageal stenosis, and hysteria, are not normal subjects for studies in metabolism, and many secondary factors exist to intensify the influence of fasting.

During the acidosis of acute starvation there is increased excretion of calcium and phosphoric acid, the fat in the blood is sometimes increased (Cohnstein, Michaelis), but the alkalescence and carbon dioxide content have been found practically normal.

The urinary ammonia usually runs parallel with the acidosis. In a muscular man with gastric ulcer von Noorden found 8.6 gm. of total nitrogen, of which 18 per cent. was excreted as ammonia. Brugsch found 35.3 per cent. of ammonia nitrogen (1.46 gm.) in one day of Succi's fast, and an average of 21.3 per cent. (1.4 gm.) from the twenty-third to the thirtieth day. Higher ratios usually belong to cases like Nebelthau's (66 per cent.), in which the total nitrogen is very low. According to Bonninger and Mohr, the ammonia excretion in fasting is far from sufficient to neutralize the oxybutyric acid in the urine, so that fixed alkalies are from the first required for this purpose. Yet Brugsch obtained entirely opposite results with Succi.

A remarkable case of chronic starvation without acidosis is reported by Brugsch of a woman of 56 years, weighing 32 kg., extremely emaciated from esophageal stenosis, who had taken no food by mouth for nineteen

days before death after gastrostomy, and who showed complete absorption of depot-fat. The urine gas gave no trace of acidosis and the ammonia nitrogen before the operation was 0.15 gm. or 2.9 per cent. of the total nitrogen (5.46 gm.). This observation does not support the idea of a toxic origin of ammonia in starvation, but it does show that starvation without fat consumption may be free from acidosis.

Starvation acidosis is not limited to patients abstaining from all food, but very marked grades commonly result from exclusive meat-fat diet. Yet in complete starvation the acetone compounds tend to increase, while on meat diet the body may learn to burn fats without carbohydrates or to appropriate the carbohydrate groups of proteins; and then the acidosis diminishes.

According to Marum, in fasting phloridzin-poisoned dogs, acetonuria appears only after the liver has become free from glycogen, and disappears coincidently with the reappearance of glycogen in the liver. Very large amounts of protein in carbohydrate-free diet tend to reduce acidosis. Dogs are more accustomed than man to lack of carbohydrate and are able to burn fats without their aid. Hence, in these animals partial starvation causes little or no acidosis so long as nitrogen equilibrium is maintained. Even in depancreatized or phloridzin-poisoned dogs there is no acidosis until negative nitrogen balance indicates the destruction of body proteins for the supply of energy (Baer, Brugsch and Bamberg).

Gerhardt and Schlesinger subjected themselves to a rigid meat and fat diet for eight days. Acetone was abundant on the first day, diacetic acid appeared on the second, and, on the fifth and seventh days, oxybutyric acid, of which 9 gm. were excreted. They reported no symptoms of intoxication.

It is characteristic of the acidosis of starvation that it is quickly relieved by carbohydrate, the administration of 100 to 120 gm. of sugar reducing the acetone in a few hours and bringing it to the normal usually within two days. Waldvogel kept a strong man four days on 1½ liters of beer and 750 gm. of white bread without increase of acetone, and Rosenfeld saw no acetonuria in a subject taking nothing but 145 gm. of cane sugar in one day. Hence, it is not the lack of food, but the absence of carbohydrates which determines the occurrence of acidosis in starvation. Yet the high acidosis with meat diets and the comparatively low grades observed in emaciated starving patients bear out Waldvogel's assertion that the degree of acidosis depends on the extent of consumption of fats.

An important question in starvation is its effect on the general vitality of the organs. Is the acetonuria purely the result of disturbed chemical

reactions in the body, or are the organ cells injured by this condition and their functional capacity reduced? Numerous interesting data bear directly on this question.

Schondorff perfused the livers of recently fed dogs with blood containing the products of digestion and found considerable formation of urea; but when the liver of a starving animal was perfused with the same blood urea was found in greatly reduced amount or not at all. Schondorff concludes that the liver in starvation shows greatly reduced capacity to form urea from the normal products of digestion. Folin, however, thinks that the amount of urea formed during perfusion through the legs of the well-fed dog was too small to justify Schondorff's conclusion. Geelmuyden found that in men on carbohydrate-free diet or fasting, 5 to 6 per cent. of diacetic acid ingested fails to be broken up, but in the same subjects on mixed diet only 0.75 per cent. escapes destruction.

While a very small quantity of carbohydrate (80 gm. glucose, Hirschfeld) is sufficient to prevent acidosis, according to Satta much larger amounts are needed to relieve an already established acetonuria. While the urinary ammonia increases with the acidosis in starvation, the observations of von Noorden (p. 51) and Satta fail to show a uniform relation between the ammonia nitrogen and the grade of acidosis in fasting. Satta shows clearly that the administration of carbohydrates in fasting promptly relieves acetonuria while the ammonia remains high for twenty-four to forty-eight hours longer. These results indicate that the withdrawal of carbohydrates causes a disturbance of cellular vitality with alteration in intermediary nitrogenous metabolism, increased excretion of ammonia, and imperfect combustion of fatty acids.

In observations on a professional faster, Cathcart found the residual nitrogen low (0.15-0.65 gm., 1.09-6.83 per cent.) and with a tendency to decline. Brugsch also obtained a low proportion of residual nitrogen in a professional faster. Yet in many diseases in which inanition is prominent, especially in the toxemia of pregnancy, the residual nitrogen is very much higher (Ewing and Wolf), indicating either that these are not cases of pure starvation or that starvation here takes on a toxic character. E. and O. Freund found only 56 per cent. of urea nitrogen on the twenty-first day of a fast, and they assume that the residual nitrogen made up the bulk of the remainder. Yet for the total nitrogen excretion of this case, 2.84 gm., this proportion of urea is normal, and much of the remaining nitrogen must probably be credited to creatin and creatinin, which were not estimated.

With deficient functional capacity of the liver the considerable excretion of indol and phenol from intestinal putrefaction becomes of con-

siderable importance as indicating a possible source of secondary toxic agents in starvation (cf. Baumstark and Mohr).

Albuminuria is of very common occurrence in starvation and points to an injury of the renal cells. Von Noorden observed a case with traces of albumin on the third day of fasting. On the fourth day broth and four raw eggs were taken. Marked albuminuria followed, lasting twelve hours. Next day, on full mixed diet, albumin was absent and later when broth and seven raw eggs were taken, it failed to appear. Von Noorden regards this observation as proving that starvation injures the renal epithelium.

The pathologic anatomy of starvation forms a very scant chapter in the extensive literature of this subject. Intestinal hemorrhages have often been observed in man and animals, and, as in Schulz's experience, fasting dogs sometimes suddenly collapse and die with intestinal hemorrhages, signs of severe intoxication, and increased nitrogen output. Delafield has long recognized in New York cases of vagabondism and starvation in which, with remarkable lack of ordinary fecal matter in the intestine, there is pronounced hemorrhagic gastro-enteritis. I have always regarded these cases as illustrating a form of autointoxication terminating starvation.

It is a widely prevalent impression that the withdrawal of food may be permitted with safety, prolonged with considerable impunity, and its consequences disregarded or estimated as a mere passing inconvenience which may be terminated at will. The study of metabolism, however, sharply contradicts this opinion, showing that starvation does not merely entail a quantitative reduction of energy, but may profoundly disturb the entire course of metabolism, lead to secondary changes in the structure of organs, and initiate a progressive autointoxication from which recovery is often slow and sometimes impossible. The observations on professional fasters can not serve as a standard for the effects of starvation in disease. Clinical experience has long established the danger of withdrawal of food in infants and children, has always recognized obese subjects as bad risks in acute infectious diseases, appendicitis and other surgical affections requiring anesthesia and operation, and has placed overnutrition in the front rank of predisposing causes of fatal autointoxication. In all these conditions it is evident that the sudden burning of body fats may constitute a serious danger to life, from loss of control of the manifold chemical reactions concerned in the process.

ACIDOSIS IN PREGNANCY.

Acidosis in disorders of pregnancy first came into prominence as a diagnostic sign of fetal death. Vicarelli in 1893 examined the urine in

137 cases of gestation and found acetonuria in nine; all these patients were delivered of macerated fetuses. He naturally concluded that acetonuria was a diagnostic sign of fetal death and finding the acetone in the liquor amnii he supposed that it must come from the fetal tissues. He did not examine the urinary distillate and when this was done by Knapp, Couvelaire, and Mercier and Menu, it appeared that acetonuria occurred in many normal cases, especially at term, was more frequent with various complications before and after labor, and was especially pronounced in eclampsia, and with some but not all cases of fetal death. Couvelaire considered acetonuria a sign of autointoxication.

Scholten, in a study of 33 cases, observed acetonuria in 31 at the puerperium, increasing with the length and severity of labor. Markedly increased acetonuria occurred during pregnancy in 3 of 39 cases, all with living fetuses. Scholten tried to bring the acidosis of pregnancy in line with the prevailing theory of origin of acetone from proteins during carbohydrate starvation, and in 9 cases he administered 100 to 300 gm. of sugar. In 6 acetonuria promptly disappeared, while in the other 3 the women vomited the sugar. Stolz found acetonuria more common in the puerperium than earlier in gestation (38 to 50 per cent.), more common in multiparæ than in first and second pregnancies. It was not favored by lactation. He thought the acetone was derived from the metabolism of body fats and was in some way connected with the absorption of colostrum. It is remarkable that these observers failed to emphasize the toxic element which must have been present in many of their cases. Yet Waldvogel, the chief exponent of the theory of origin of all acidosis from carbohydrate starvation, detected certain discrepancies between the acetonuria of pregnancy and that of pure malnutrition.

The autotoxic nature of the disorders of pregnancy accompanied by acidosis was virtually proved by the observations of Lindemann and Bouffe St. Blaise, but the close relation between all these specific manifestations was first shown by Stone and by the writer of this paper in 1903-4. At that time we had found high ammonia ratios in the urine in pernicious vomiting, indicating severe acidosis, but finding no constant relation between the acidosis and the severity of toxic symptoms it became necessary to look to other features of metabolism for a clue to the nature of the intoxication.

Meantime, Zweifel reported an extensive study of the urinary sulphur in eclampsia, concluding that this disease consists in a remarkable deficiency in the oxidative capacity of the organism, and attributing the toxic symptoms chiefly to sarcolactic acid. According to this view, eclampsia falls in that group of acidoses which is antagonistic in origin to that due to the acetone compounds.

Williams has contributed important observations on the ammonia excretion in pernicious vomiting, dividing the cases into two groups, those with high ammonia, which he recognizes as toxic, and those with low ammonia, which he regards as neurotic.

In a study with Wolf of the clinical symptoms, pathologic anatomy, and urinary chemistry of the toxemia of pregnancy, we drew the conclusion that pernicious vomiting, acute yellow atrophy and eclampsia are closely related conditions, connected by transitional cases, and consisting essentially in a disorder of nitrogenous metabolism involving disturbances of several processes, including probably defective desamidation. From the results of this work the routine study of the nitrogen partition in the urine in pregnancy was recommended, partly as a reliable guide to the gravity of the disease, but more urgently as a basis for prophylactic treatment by general hygiene and carefully adjusted diet. Although we found acetone compounds in many cases and recognized the significance of ammonia as a measure of acidosis, we were unable to regard the disease as essentially an acid intoxication, since these signs were missing in many very severe cases of all types, while the low urea and high amido-acid nitrogen in such cases suggested the theory of defective desamidation.

Quantitative estimation of the acetone compounds in the toxemia of pregnancy are not available, but from the ammonia ratios reported by Stone, Williams, Edgar, Ewing and Wolf, in pernicious vomiting, and by Zangemeister and Zweifel in eclampsia, it is evident that the acidosis is often severe. In one of our cases after two weeks vomiting the total ammonia nitrogen was to 1.78 gm. (14.2 per cent.); and in another case, that of a very fat woman with moderately severe symptoms, the ammonia nitrogen was 43 per cent. It is especially in the acute cases in the early months that high ammonia ratios occur. No uniform rule can be established, however, owing to the complexity of factors influencing ammonia excretion. We have seen fatal cases with normal ammonia, and are now observing a patient whose ammonia ratio for some months has run between 10 and 15 per cent. in spite of full carbohydrate diet. This patient was finally delivered of a healthy infant, after which the urine promptly became normal.

Recently a remarkable case of pernicious vomiting came under our observation at Bellevue Hospital through the kindness of Dr. Cyrus J. Strong. This patient, a well-nourished teripara, had taken little food for some weeks on account of persistent vomiting. Her condition did not appear to be critical, the chief symptoms being muscular weakness, mental dulness, and vomiting. The pulse was 80 to 100 and of good force. The accompanying table of urine analyses is furnished by my colleagues, Drs. Wolff and Shaffer.

On July 8 abortion was recommended, the patient was transferred to another service, and no further analyses were obtained. Instead of operation, moral suasion was employed with much success, as the patient stopped vomiting and retained some food. On July 14 she was delivered of a macerated four months fetus. Vomiting recurred severely and the patient sank into a state of great weakness, mental dulness, delirium ending in coma and death, July 20. There was no autopsy.

In this case the remarkable ammonia nitrogen ratio of 75 per cent. (total 2.47 gm.) was reached and urea disappeared entirely from the urine of this day. On account of the very low nitrogen output the chemical diagnosis must be chronic starvation with acidosis, but clinically the termination of the disease took the form of pernicious vomiting of pregnancy with acute yellow atrophy of the liver.

In many of our cases, however, the ammonia totals and ratios are normal or subnormal, although the symptoms are severe or even fatal (cf. Cases 10, 12, 16-19, Ewing and Wolf). Hence, acidosis is not a constant and can not be an essential feature in all stages of the toxemia of pregnancy.

In eclampsia the ammonia ratios of Zangemeister and Zweifel, as well as our own, run below 20 per cent., while the total ammonia nitrogen does not indicate the existence of a severe grade of acidosis, and the reactions for acetone compounds are moderate.

Zweifel believes that eclampsia is an intoxication by lactic acid or its salts, but the amounts of this acid which he finds in the blood and urine are comparable to those found in other quite different conditions, while the known physiology of lactic acid does not favor the belief that it is the cause of eclamptic seizures. Dreyfuss in a recent study of eclampsia has pointed out these physiologic relations and concluded that in eclampsia lactic acid results from excessive muscular exertion, dyspnea, and disturbance of the liver.

That the specific toxemia of pregnancy is an autointoxication is amply proved, since its fulminant forms are the most violent occurring in the human subject; and it is almost equally clear that acid intoxication is a subordinate factor, although possibly affecting some cases, but the part played by starvation in this disease is not easily determined.

In the case here reported and in Case 12 of Ewing and Wolf's series the influence of prolonged starvation seems evident. But more rapid cases of the same type may be fatal in a few days, and if these are the result of starvation, then in this class of patients the essentially toxic character which starvation may sometimes assume becomes emphasized to a remarkable degree. Von Noorden seems to believe, and his pupil,

URINARY ANALYSES IN A CASE OF TOXEMIA OF PREGNANCY.

Date.	No.	Volume of Urine, c.c.	Albumen.	Titrated Acidity, 100 c.c. = $\frac{n}{c.c. - 10}$	Total Nitro- gen.	Gross Urea Nitrogen		NH ₃ Nitrogen		Net Urea Nitrogen		Creatinin Nitrogen		Creatin Nitrogen		Uric Acid Nitrogen		Rest Nitrogen		Remarks.
						gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	
6-11	...	770	+	slight acid.	2.56	2.77	77.5	1.83	50.9	0.95	86.6	0.11	8.1	0.13	3.4	0.06	1.7	0.03	14.5	Vomits frequently. Dull. Pulse 90-100.
6-12	173	760	+	slight.	2.44	1.67	64.4	0.99	40.6	0.58	23.8	0.19	7.8	0.08	3.3	0.04	1.9	0.13	5.3	
6-30	173	1140	+	slight.	3.33	2.43	72.8	1.77	53.1	0.66	19.7	0.265	8.0	0.033	0.7	0.04	1.4	0.07	17.1	
6-31	176	1085	+	trace.	3.34	2.41	72.2	1.79	53.6	0.68	18.5	0.290	8.0	0.034	0.7	0.04	1.3	0.07	18.0	
6-33	177	1440	+	slight.	3.60	2.84	76.5	2.81	62.7	0.58	12.8	0.28	7.6	0.04	1.1	0.09	2.4	0.44	12.0	
6-33	178	1175	+	slight.	3.33	2.56	77.0	2.47	74.2	0.09	2.8	0.21	6.4	0.06	1.9	0.10	2.9	0.40	12.0	
6-34	179	1410	+	slight.	2.75	2.17	75.0	2.17	62.5	0	0	0.193	7.0	0.045	1.7	0.103	3.7	0.35	12.7	
6-35	180	1380	+	slight.	3.38	2.64	78.3	2.13	62.5	0.51	15.8	0.167	5.0	0.12	3.6	0.13	3.9	0.32	9.5	
6-36	181	1300	+	slight.	2.58	2.08	78.7	1.75	67.8	0.28	10.9	0.184	7.1	0.08	1.3	0.066	2.5	0.37	10.5	
6-30	184	870	+	neutral.	1.33	0.77	60.9	0.354	27.8	0.42	32.1	0.17	13.3	0.043	3.4	0.016	1.3	0.38	22.7	
6-30	185	885	+	slight acid.	4.05	2.86	70.6	0.71	17.5	2.15	63.1	0.33	8.1	0.06	1.6	0.17	4.1	0.63	15.6	
7-1	186	860	+	slight acid.	4.45	3.63	81.5	0.49	11.0	3.14	70.5	0.223	5.0	0.05	1.3	0.104	2.3	0.37	9.0	
7-2	188	200	+	slight acid.	1.81	1.50	82.9	0.16	8.9	1.34	74.0	0.079	4.4	0.026	1.4	0.031	1.7	0.17	9.4	
7-3	189	435	+	slight acid.	3.43	2.78	81.3	0.30	8.7	2.43	73.3	0.157	4.6	0.011	0.3	0.063	1.5	0.41	12.0	
7-8	190	230	+	acid.	2.65	2.05	77.5	0.14	5.3	1.39	72.3	0.167	6.3	0.034	1.3	0.033	2.3	0.34	12.7	
7-11	201	455	+	acid.	4.5	3.30	73.3	0.365	5.7	3.04	67.7	0.313	7.1	0.037	1.9	0.14	3.1	0.66	14.7	

Satta, actively maintains, that the condition in fasting is essentially toxic with progressive injury to the body cells, and such is the conclusion reached in the present section on this topic.

It has been objected to the use of the nitrogen partition as a clinical guide in the toxemia of pregnancy, that it shows only the influence of starvation. According to the present view, however, its significance is not thereby reduced, since it would then indicate the progress of a type of starvation which may prove rapidly fatal. But it is difficult to accept the peculiar symptoms of pernicious vomiting as those of starvation, and the urinary analyses are in some important respects quite different.

Among the chief distinctions are the inconstancy of acidosis, the occurrence of high total ammonia without acidosis, and the high proportion of amido-acid nitrogen. These signs point to a deficiency of urea forming function, possibly to defective desamidation, leading to degenerative changes in the liver and other organs and to a fatal collapse of the chemical control of the organism. The clinical condition shows a striking resemblance to that described by Minkowski after extirpation of the liver or following the Eck fistula. According to this view, which assumes the existence of injured organ cells, there is room for the belief that an excessive neurotic element or severe acidosis or indol poisoning, may stamp certain cases with peculiar features. It is probable that many of the fatalities in this case are due to delayed chloroform poisoning. Yet obstetricians continue to use this anesthetic freely.

It is not an infrequent experience to find that persistent vomiting in the earlier months of pregnancy is followed after an interval by albuminuria and later by the pre-eclampsic state of Edgar, or by eclampsia with moderate or pronounced nephritis. The observations on acidosis threw some light on this sequence of events. In many experimental forms of acid intoxication beginning with the discovery of Kulz's coma casts, which I have observed in hydrochloric acid poisoning, in the experiments on the toxicity of acetone compounds, and in many clinical studies, it is apparent that prolonged acidosis tends to set up nephritis. Diabetes for special reasons is a partial exception to this rule, and yet it is extremely rare for diabetes to prove fatal without active nephritis. Of the mechanism of this relation little is known, but the fact that it exists is sufficiently attested and should encourage efforts to combat acidosis whenever it appears, especially in the toxemia of pregnancy.

CYCLIC VOMITING.

There are several types of periodical vomiting in children and in one of these acidosis is prominent and acid intoxication has been urged as the

essential pathogenic factor. There is a purely neurotic vomiting described by Leyden, Raymond and others; a second type, referable to errors of diet and relieved by the vomiting and purging, is described in this connection by Fenwick, Symes, and Gee, and it is obvious that any of the bacterial causes of gastritis in children may act periodically.

Characteristic cyclic vomiting occurs only in children, usually in neurotic subjects, at frequent intervals, not connected with dietetic errors, and not relieved by vomiting or purging. The vomiting is very severe, and the vomitus may contain mucus, bile, blood, acetone, and indol, and is accompanied by great prostration, thirst and emaciation, but not by pain. There is restlessness, headache, and there may be delirium, convulsions and coma. The temperature may be subnormal or rise to 110 F. Respiration is rapid, sighing and irregular. The attack usually lasts but a few days, but may be continued for two weeks or prove fatal in forty-eight hours. Recurrence is common. The urine before the attack may contain much indican, increased uric acid, and some acetone; during the attacks it is diminished with less indican or uric acid, more acetone compounds, and often with albumin, casts, or blood.

Several theories of the nature of this disease have been maintained. The theory of a gastric neurosis applies to one predisposing element. Rachford believes that the malady is a lithemic condition allied to migraine, and in one of his patients the attacks changed to migraine as the patient grew older. Excess of uric acid has been found in the urine by Pepper, Holt, Griffith, Valagusa, and Comby, and appears to be a very constant feature of the disease.

The acetone compounds have been found in the urine and breath by Marcy, Edsall, Marfan and others, and Edsall has urged that the disease is a form of acid intoxication.

Several observations favor this hypothesis. Although quantitative estimations of the acetone compounds have not been made, the reactions in the urine have often been pronounced, and the breath may give a strong odor of acetone. Acetone may occur in the urine before the attack (Marfan). It may appear in the vomitus, suggesting that the vomiting is an eliminative mechanism. Edsall states that the respiratory disturbance resembles that of diabetic coma and he strongly recommends alkali therapy, while he and Pierson assert that they have cured some and prevented other attacks by this method. On the other hand, the acetone compounds do not appear to be present in quantities sufficient to account for the severe symptoms, and their toxicity is slight. These substances occur very readily in children not presenting the symptoms

of cyclic vomiting (Baginsky, Langmead). The resemblance of the dyspnea to that of acid coma has not impressed the majority of observers. The good effects of alkali therapy here, as in diabetic coma, may be referable to other actions than the neutralization of acids (Shaw and Tribe), while this treatment may fail and other forms of treatment may be equally or more effective (Griffith, Shaw, Tribe and Marfan).

The researches of Howland and Richards have gone far toward elucidating the nature of cyclic vomiting in children. In a series of cases they found acetone compounds and lactic acid, a rise in the ratio of neutral to oxidized sulphur, and heavy indicanuria, which diminishes during the attack. All these urinary signs they interpret as evidence of deficient oxidation by the body cells. They argue that the rise in uric acid must represent endogenous nucleo-proteid metabolism since exogenous sources of uric acid are reduced with the scanty diet of the patients.

The appearance of acetone compounds and lactic acid can not be due, they think, to lack of carbohydrates, since the symptoms appear too soon—three to four hours after a full diet—and must be referable to failure to burn carbohydrates and fats properly. They attach great importance to the presence of much indol, phenol and skatol in the urine. Before the attack indicanuria is greatly increased, indicating increased intestinal putrefaction. While indol is comparatively non-toxic in the healthy organism, they show that when the animal's detoxicating power is reduced by slight poisoning with potassium cyanid or chloroform, or by asphyxia, indol becomes extremely toxic, producing vomiting, hemo-mesis, blindness, convulsions, and death. In dogs thus poisoned by potassium cyanid 0.25 to 0.5 gm. of indol produced very marked symptoms, although 18 gm. of indol have been found innocuous in healthy animals. In their poisoned dogs the excretion of indol as indican was delayed and less was found in the urine than with normal dogs. This observation indicates that the amount of indican in the urine is determined not merely by the amount of indol absorbed, but by the capacity of the organism to combine with sulphuric acid and excrete it as indican. I have repeatedly found that indican decreases or completely disappears in the urine, during attacks of migraine, in patients who excrete large quantities before and after the attack.

Howland and Richards conclude that cyclic vomiting results from deficient oxidation brought about in predisposed subjects by nervous disturbance, and leading to failure to detoxicate products of intestinal putrefaction, and of internal metabolism.

That the subjects of cyclic vomiting suffer from severe autointoxication is indicated not only by the urinary signs, but by the extensive gran-

ular and fatty degeneration of the liver and kidneys recorded in the autopsies of Griffith and Marcy, and observed in many other conditions accompanied by acidosis.

There is also good reason to believe that indol is a prominent toxic agent in this disease. Since similar evidence has not been obtained of the toxic action of acid products of metabolism, and since the quantity of these acid products excreted has not been determined, and since the symptoms and lesions of the disease are not those of the most probable manifestations of simple acidosis, I think one can not endorse the view that cyclic vomiting is a form of acid intoxication, with extensive loss of tissue alkalies. It appears to be rather a complex disturbance of metabolism occurring in neurotic and predisposed subjects, in which rapid burning of body fats, defective function of the liver, and poisoning by intestinal putrefactive products are combined. Studies on the nitrogen partition, especially the ammonia and residual nitrogen, quantitative estimations of the acetone compounds, of lactic acid, and of the alkalescence of the blood are needed in this field, and are essential before one can estimate the relative importance of these factors.

ACIDOSIS AFTER ANESTHESIA.

Becker, who first studied the acidosis following anesthesia, found acetonuria in two-thirds of all subjects. It was more marked in women than in men and most pronounced in children, appearing in the first or second portion of urine passed, and lasting eight or nine days. It was uninfluenced by the length or character of the narcosis, and occurred after one minute's inhalation of bromether. Abram found acetone in all of 25 cases, but in 9 Legal's reaction appeared only in the distillate. Ether seemed to be less effective than chloroform. Waldvogel observed acetonuria in three-fourths of fifty cases, demonstrating diacetic and beta-oxybutyric acids in thirteen cases on the first day. The highest amount of beta-oxybutyric acid observed was 2.5 gm. in eighteen hours. In two cases acetone compounds were at first absent and considerable glycosuria occurred.

The fatal effects of anesthesia in diabetes, of which Becker collected thirteen cases, seemed thus to receive a partial explanation, since it was early seen that existing acetonuria was made worse by the anesthetic. A direct toxic origin of this form of acetonuria seemed obvious, but Waldvogel attempted to prove that it was chiefly referable to the withdrawal of food in the preparation for the anesthetic, and to the subsequent vomiting. In several cases he was able to show that acetonuria did not appear if the patient was anesthetized on a full stomach and did

not vomit after recovery. Yet, since children of 6 to 9 years, whom he allowed to fast for seven hours, failed to show increased acetonuria, and since the influence of carbohydrate in relieving the acidosis from chloroform was much slower than in simple starvation, Waldvogel was forced to conclude that a direct toxic action of the anesthetic was an important factor in producing the acidosis. Baldwin, in a study of forty-one cases, gave 25 to 50 gm. of glucose to six patients without evident effect on the acetonuria.

It has been shown in dogs (Marum) and assumed for man that acetone does not appear in the urine until the glycogen of the liver has been consumed and no more carbohydrate is available. But the immediate appearance of acetone after anesthesia shows that this course of physiologic events is not invariable and points to a direct interference with carbohydrate consumption exerted by the chloroform. The very early appearance of fatty degeneration of the liver after chloroform narcosis is of similar import.

It has long been known that the dangers of chloroform, and, to a less extent, of ether are not exhausted in its immediate effects. In recent years attention has frequently been drawn to the occurrence, chiefly in children, of a type of delayed chloroform poisoning, in which the patient after more or less complete recovery from the anesthetic, after a few hours or days, fell into a state of fatal intoxication marked by vomiting, acetonuria, acetone breath, extreme prostration, albuminuria, cyanosis, nervous excitement, followed by delirium, coma, and death.

Guthrie, in 1894 and again 1903, emphasized the frequency and importance of this peculiar intoxication, regarding it as a result of chloroform poisoning added to a pre-existing fatty degeneration of the liver. So pronounced was this change in the liver and so much did the symptoms resemble those of acute yellow atrophy, that he was inclined to believe that the two conditions were closely related, and he referred to cases of acute yellow atrophy following operation collected by Schenck and by Ballin in support of this view.

That these cases may be accompanied by marked acidosis was shown by Brewer in 1902 in a remarkable case fatal three days after operation for acute appendicitis, in which considerable amounts of acetone and diacetic acid were found.

Bracket, Stone and Low, and later Kelly, have reported a series of cases of this general type occurring in young children at the Boston City Hospital. The patients were, as a rule, rather poor subjects for surgical treatment, some suffering from infantile paralysis or intestinal obstruction; one seems to have been subject to cyclic vomiting, and all

exhibited a neurotic tendency which was not improved by hospital surroundings. In six cases the symptoms developed without operation, and one of these was fatal, but most of the severe cases occurred after anesthesia by ether. The grade of acidosis was probably not severe, as Joslin found only 0.142 gm. of acetone in the urine from a patient dying twenty hours after operation. At autopsy extensive fatty degeneration of the liver and kidneys was found. Treatment by fat-free diet and bicarbonate of soda seemed to have good effects in some cases, but not in others, and no better than those following salt infusion. Later Stiles and McDonald (1904) presented a full clinical and pathologic picture of delayed chloroform poisoning, assuming that it is a form of poisoning by acetone compounds; and a similar conclusion was reached by Carmichael and Beattie, the latter authors excluding *constitutio lymphatica* in their cases. Bevan and Favill, in a full review of the subject, cite several cases of acute yellow atrophy of the liver following anesthesia, in which group their own case seems to fall. They strongly emphasize the dangers of chloroform anesthesia in predisposed subjects and conclude that the chloroform directly injures the liver cells while the acidosis is a secondary and comparatively unimportant feature of the intoxication.

There is no doubt that the cases included in the foregoing reports represent a heterogeneous group of conditions, of which acidosis is a more or less constant feature. None of the authors brings forward satisfactory evidence that acid intoxication of any form is a prominent factor in the symptoms, but the occurrence of this type of fatal intoxication is not only of great practical importance, but has rather decisive bearing on the general question of the significance of acidosis and acid intoxication. These cases demonstrate a highly toxic condition with extensive injury to organ cells, in which there is marked acidosis, and thus they emphasize the toxic element in some other forms of acidosis. They leave undetermined, as it appears to me, what part, if any, the acidosis plays in the symptoms and fatal issue, but many of the cases suggest that the sudden burning of body fats when the liver is seriously injured is a process that may become dangerous to life. Yet the observations are quite inadequate to prove the real character of the underlying disturbance of metabolism.

In some cases of delayed chloroform poisoning and of acute appendicitis fatal after operation I have found very low urea, rather high ammonia and excessive residual nitrogen, suggesting that this is a condition in which the urea-forming and other functions of the liver are fatally deficient. Further systematic urinary analyses are essential for the correct interpretation of this group of cases. There are many reasons for supposing that it will be found to follow the prototype of the intoxication following extirpation of the liver, or the Eck fistula.

From the practical side the observations on delayed chloroform intoxication are of much value, since they serve to define an important particular in which certain subjects may be recognized as bad surgical risks. It is the consensus of opinion, from which only Carmichael and Beattie dissent, that the intoxication occurs only in predisposed subjects of nervous temperament, and weakened by fatty alterations of the liver, and one need not assume the existence of fatty degeneration in order to explain the weakened state of the organism. In view of the many unfavorable factors, as youth, previous chronic malnutrition or disease, and anesthesia, which seem to concur in producing the fatal result, it may be necessary to reopen, for this group of cases, the question of the direct toxicity of salts of the fatty acids. In any event attention may be called to this group of cases as a most favorable field for the study of problems relating to acidosis.

MISCELLANEOUS CLINICAL TYPES OF ACIDOSIS.

There remain for consideration several clinical conditions in which von Jaksch believed he could recognize the effects of acetonemia, but in which later investigations have shown that the acidosis is the result and probably not the cause of symptoms and that the acetonuria is not the result of absorption from the intestinal tract, but the sequel of carbohydrate starvation. Under the title of "acetonuria from digestive disturbances" have been described a heterogeneous class of cases, marked by vomiting and diarrhea, acetonuria, and a variety of nervous symptoms which the older authors were disposed to refer to acetonemia. In this field it is extremely difficult to attempt any classification or to trace the influence of many factors concerned, but several somewhat coherent groups of cases may be recognized.

One of these has been separately considered as "cyclic vomiting of children." In addition to this somewhat special recurrent type, the ordinary gastro-enteritis of infants has been found associated with the presence in the urine of all the acetone compounds, and the nervous symptoms of many of the cases suggests a relation to acetonuria (Vergely, Schrack, Engel). In the extensive reports of von Jaksch and of Lorenz one finds many cases of digestive acetonuria with epileptiform convulsions, which, on inspection, one must attribute to a great many different diseases, as eclampsia, uremia, lead poisoning, hydrocephalus, chronic meningitis, etc.

Cassaet has isolated highly toxic alkaloids from the stools of such patients showing much acidosis, and it is much more reasonable to attribute the symptoms to this class of substances, rather than to any form of acidosis.

COMA DYSPEPTICUM.

"Coma dyspepticum" is a term applied to a group of symptoms described by Litten and observed by him in five cases. After a short period of dyspepsia referable to errors of diet the patients began to suffer from prostration, chilliness, anorexia, thirst, constipation or diarrhea, and occasional vomiting soon followed by pronounced nervous disturbance, headache, restlessness and excitement varying with depression. Later a pronounced odor of acetone on the breath, and marked ferric chlorid reaction in the urine appeared, and the patients became dull, apathetic, and semicomatose. After two or three days recovery followed with disappearance of the acetone. Although glycosuria was absent, Litten regarded this condition as related to diabetic coma. The relation of acetonuria to coma dyspepticum appears never to have been determined.

ASTHMA ACETONICUM, HYSTERIA AND OBSTIPATION.

The "asthma aceticum" of Pawinski has long been recognized as uremic vomiting and nephritic dyspnea.

In hysteria with vomiting high grades of acetonuria may obviously be attributed to starvation, but the process may nevertheless be toxic. In cases of obstipation very strong acetone reactions have been obtained in both urine and feces, but nervous symptoms such as might be attributed to acetonemia are inconstant or absent (cf. Lorenz, case 37).

In all the above groups the lack of relation between the nervous symptoms and the grade of acetonuria, the appearance of acetone after and not before the onset of symptoms, and the existence of many other probable sources of nervous symptoms, strengthen the belief that the acetonuria is the result chiefly of carbohydrate starvation and that the acidosis is usually a secondary and negligible factor.

The possibility that acetone compounds absorbed from the intestine contribute to the symptoms has been frequently mentioned (Vergeley), but is not strengthened by the relatively small amounts of these substances so far demonstrated in the gastrointestinal contents (Savelieff, Baginsky), while any possible toxicity of the fatty acid derivatives is very far below that of indol and other putrefactive products. The fatty stools of milk-fed infants have perhaps not been sufficiently studied from this point of view. Excess of fat in the food increases acetonuria, and carbohydrates diminish intestinal putrefaction (Hirschler). Hence, there is perhaps still some reason to believe that in some cases acetonuria in digestive disturbances may be partly referable to intestinal absorption.

ACIDOSIS OF CANCER.

Among the types of acetonuria described by von Jaksch was that occurring in the late stages of gastric carcinoma in certain well-nourished patients who suffered from severe vomiting. Some of these patients passed into coma and it was pointed out by Riess and Litten that sufferers from this coma often exhibited the "grosse Atmung" of Kussmaul's dyspneic coma of diabetes. Von Jaksch stated that these patients were not always suffering from starvation; that the acetonuria might appear unexpectedly in the disease; and that its appearance sometimes marked the onset of a severe type of cancerous cachexia.

In two cases of esophageal and gastric carcinoma with typical Kussmaul's coma, Klemperer demonstrated a daily loss of nitrogen on balance of 5 to 9 gm., indicating toxic destruction of tissues, and there were also small amounts of beta-oxybutyric acid in the urine. Klemperer concludes that in both cancer and diabetes the coma, the destruction of tissues, and the acidosis, are the result of a general toxic process, but that in neither case does the acidosis produce the coma. F. Müller had previously shown that nearly all cases of cancer lose nitrogen on balance, and Waldvogel suggests that destruction of protein tissues may very well be associated with toxic destruction of fats and thus give rise to acidosis.

It is evident that in most cases of cancer coma the patients are undernourished or starving and that inanition is the chief factor in this form of acidosis. Von Noorden finds that acetonuria is absent in cancer when the nutrition is good and begins to appear when the destruction of proteid tissues becomes evident. Waldvogel failed to find increased acetonuria in several advanced cases of cancer in which the patients were well fed, and Hirschfeld relieved the acidosis in such cases by giving sugar. It nevertheless appears possible that the extensive burning of body fats and proteins in a cachectic organism may be one of the factors responsible for some of the sudden terminations of carcinoma.

ACIDOSIS IN PSYCHOSIS.

The prominence of nervous symptoms lends special interest to the acetonuria of psychoses, yet in this field there has never been a strong tendency to refer the nervous symptoms to acetone poisoning or acid intoxication. Tuzcek first observed diacetic acid in the urine in psychoses when the patients were fasting, while von Jaksch was unable to prove that acetonuria was at all constant when such patients were well nourished. Jauregg, however, asserted that he was able to detect peculiar characteristics in certain psychoses which showed a definite relation to acetonuria, which he regarded as of intestinal origin, and for which he recommended

intestinal antiseptis. That no such relation exists in melancholia, mania and paresis was fully shown by de Boeck and Slosse, who found acetonuria in such cases only when the patients were inadequately fed. In epilepsy acetone may appear after severe seizures, together with lactic acid (Luthje, Araki).

FEBRILE ACETONURIA.

In the extensive literature on this topic one finds further illustration of the rules governing the occurrence of acetone compounds, but no indication that acidosis is a necessary or significant feature of the febrile process or that it contributes to the common symptoms of infectious diseases. Von Jaksch found that it usually—but not always—bore a relation to the height of the fever, but it was inconstant in occurrence, reaching a grade (maximum 500 mg.) considerably lower than that of starvation or diabetes and without definite relation to prognosis. In children, as usual, it becomes most pronounced. In pneumonia of children Pfaundler observed acetonuria in twenty-nine of fifty cases. In adults Engel, in five cases, saw moderate but variable increase. Lambert and Wolf, in a series of cases, report rather pronounced increase in the total ammonia, indicating considerable acidosis.

In typhoid fever the acetonuria is more marked and constant but by no means invariable. Engel found a maximum of 226 mg. acetone in seven cases. In two of these acetonuria and diarrhea were absent. Fraenckel frequently saw initial acetonuria disappear when a moderate amount of food was given. As judged by the ammonia nitrogen in a series of cases I found typhoid fever to be strikingly free from acidosis. Even with an antemortem fall in net urea the ammonia did not rise above 10 per cent. and usually remained within normal limits. The occurrence of very toxic cases of typhoid fever and of terminations in acute yellow atrophy show that this disease may be complicated by severe types of autointoxication (Ewing), and among the factors concerned may perhaps be found severe acidosis from burning of body fats and decline of hepatic function.

In measles and scarlet fever Kulz early detected beta-oxybutyric acid. In a case of scarlatina in a woman of 31 years Litten describes a typical case of Kussmaul's coma with marked acetonuria which disappeared with relief of the coma.

That the element of partial starvation is concerned in febrile acetonuria is conceded by all observers, but many have expressed the opinion that a toxic destruction of tissues is also to be considered as a factor. Botazzi and Orefici show that in diphtheria the acetonuria reaches its maximum at the time of greatest loss of nitrogen balance and

is promptly reduced by antitoxin. Blumenthal found in streptococcus infection a special tendency to cause acetonuria. The increased acidosis observed in diabetics suffering from febrile disease points to a toxic process (Ebstein, Engel). The carbon dioxid content of the blood is greatly reduced (Loewy, Münzer, Meyer) and the titratable alkali is much diminished, 35 mg. sodium hydrate (von Jaksch).

After a full consideration of the complex conditions in which febrile acetonuria appears, Waldvogel concludes that it results from destruction of fats due to inanition and cell injury and is influenced by the nature of the poison, the location of the disease focus, the instability of the fat tissues and individual peculiarities.

ACETONURIA FROM POISONING BY DRUGS.

Varying grades of acetonuria are observed after administration of many drugs, as phosphorus, arsenic, lead, phloridzin, antipyrin, pyrodin, morphin, atropin, curare, carbon monoxid and sulphuric acid. In the action of all these there appears to be an influence of starvation, but also a more prominent toxic effect. In some instances the toxic influence seems to act through deficient oxidation, as with the hematoxic agents, pyrodin, carbon monoxid and with phosphorus. With all of these the acetonuria appears very promptly, may be increased by larger doses of the drug, and as a rule is comparatively uninfluenced by any carbohydrate that may be given (Walko, Boeri, Waldvogel). In phosphorus poisoning acidosis appears in the first hours but is not always proportional to the severity of the intoxication or to the glycosuria. Schwarz found in breath and urine 0.61 mg. acetone on the third day, 0.56 gm. on the second day, mostly in the breath, while a larger amount, 1.31 gm., was determined on the second day of a very mild case. Diacetic acid was commonly present, but beta-oxybutyric acid was never detected. The acidosis was not influenced by carbohydrate feeding. Münzer found the ammonia nitrogen regularly increased from 10 to 22 per cent. (0.5 to 2.2 gm). He regarded the increased ammonia as solely the result of acidosis in which sarcolactic acid was prominent, and since administration of alkalies reduced the ammonia without affecting the symptoms, he held that poisoning by ammonia salts did not occur. He usually detected acetone in marked amounts.

The exact sources of the acidosis of phosphorus poisoning have not been finally determined. It is evident that the acetone compounds are insufficient to explain it, and most authors consider lactic, phosphoric and sulphuric acids as the chief sources. Münzer calculated that the excess of urinary ammonia could all be referred to lactic, phosphoric,

sulphuric and oxy-acids derived from increased destruction of proteins. Yet the extreme disturbance of the fat depots of the body, the splitting off of fatty acids and lecithin in the organ cells (Mavraki, Saxl), and the interference with several normal metabolic processes, give abundant opportunity for the formation of acetone compounds, which Waldvogel and others believe are prominently concerned in the acidosis of phosphorus poisoning.

Phloridzin produces acidosis in all animals, least promptly in the dog. According to Baer phloridzinized dogs in nitrogen equilibrium show no acidosis; acidosis appears only when the glycogen of the liver has been exhausted and proteins and fats are being called on for energy. The demonstration of this rule governing the appearance of acetonuria has thrown much light on the mechanism of action of the toxic causes of acidosis and is further evidence of the essential relation of carbohydrate combustion to these forms of acidosis.

With the remaining drugs which induce acetonuria the grade of acidosis is moderate, and the factors of malnutrition and special toxic action are variously combined, but in none of them do the data seem to deserve special consideration at this time from the clinical or theoretical side.

***THE SIGNIFICANCE OF THE PATHOLOGIC ANATOMY OF ACIDOSIS AND
A CLASSIFICATION OF ACIDOSES BASED CHIEFLY THEREON.***

Pathologic anatomy has been given a very scant hearing in the datable ground of acid intoxication, but it has seemed to me that more important clues might be obtained from this field than have yet been secured. Indeed, notwithstanding all that may be said about the lack of relation between histologic structure and functional capacity of organs, I think it will eventually be proved a grave error to associate together such conditions as diabetic acidosis, in which the liver is practically normal, and that of delayed chloroform poisoning, in which this important organ may be nearly destroyed.

In the two experimental prototypes of acidosis, Walter's acid poisoning and Minkowski's extirpation of the liver, the anatomic conditions are entirely different. In poisoning by hydrochloric acid there are no prominent anatomic lesions, while after various procedures for eliminating the function of the liver, as the Eck fistula or injections of acid into the bile ducts, this organ becomes completely necrotic. Reviewing the clinical types of acidosis, especially their pathologic anatomy, it appears possible to separate them into two rather distinct classes resembling the two experimental types above mentioned. This division may be based mainly, in one, on the absence of anatomic changes in the viscera and

presence of excessive amounts of acetone compounds in the urine, and, for the other, on the occurrence of marked degenerative changes in the organs and the presence of much lactic acid. In the former case acids are produced in excess and ammonia is diverted to neutralize them. Here the formation of acids is the primary event, while the diversion of ammonia is a compensatory process. In the latter type ammonia appears in excess, not to neutralize acids but from failure of its synthesis into urea, while the acids result partly from failure of urea formation but also from other factors in the disease. Whatever their source, some of these acids unite with ammonia, but this fact does not prove that the process is merely compensatory.

CLASSIFICATION OF ACIDOSES.

GROUP 1.—TYPE: HYDROCHLORIC ACID POISONING.

Pathologic Anatomy: No prominent lesions.

Pathologic Chemistry: Acetone bodies chiefly present; ammonia proportional to these acids; amido-acids slightly increased.

Clinical Forms: Diabetic coma; Kussmaul's coma; starvation.

GROUP 2.—TYPE: EXTIRPATION OF LIVER, ECK FISTULA.

Pathologic Anatomy: Extensive fatty degeneration.

Pathologic Chemistry: Lactic acid abundant; acetone bodies less prominent; ammonia in excess of fatty acids; amido-acid nitrogen much increased; glucose frequently present.

Clinical Forms: Phosphorus poisoning; pernicious vomiting of pregnancy; acute yellow atrophy; eclampsia; delayed chloroform poisoning; cyclic vomiting.

The general facts on which this division is based are contained in previous discussions, but some further grounds may be briefly reviewed in its support.

In diabetic coma the urinary chemistry and the clinical symptoms obviously accord with the type. The liver in diabetic coma varies extremely, but in uncomplicated cases usually shows a striking absence of fatty degeneration. In Naunyn's series of thirty autopsies fatty liver was found but once and that in a case of spontaneous diabetes in a dog. Minkowski has collected many reports, most of which indicate that fatty livers are comparatively rare in diabetic coma. Fatty liver occurred but once, and in an alcoholic subject, among eleven cases of diabetic coma which I have recently collected. Therefore I conclude that fatty liver is not essential in diabetic coma in man, and when it occurs may usually be referred to complications.

In starvation the organs undergo a process of simple atrophy, in which fatty changes are not prominent (Morpurgo). In the earlier stages of hunger in dogs there may be a moderate deposit of fine fat

granules in many gland cells, but these disappear in the late stages (Nicolaides). Extreme grades of fatty degeneration are apparently quite unknown in simple starvation. In the urine of starvation acidosis acetone compounds are abundant and lactic acid scanty.

On the other hand, all the forms of Group 2, except eclampsia, are characterized by a remarkable grade of fatty degeneration of the liver and often of other organs. In eclampsia fatty degeneration is usually not pronounced but acute degeneration or autolysis is constant, and there is reason to regard this change as of more significance than the hemorrhagic hepatitis.

In phosphorus poisoning, acute yellow atrophy, and eclampsia, lactic acid is more prominent than the acetone compounds, but in the other diseases of this group lactic acid has not been fully studied, while the presence of acetone bodies one may refer to complicating acidosis of starvation or toxic consumption of fats.

In the second group the severity of the condition is much greater than in the first. In diabetes and starvation the metabolism of fats is defective owing to lack of carbohydrate combustion, while the structure of the organs does not suffer, but in phosphorus poisoning and eclampsia the liver and other organs are severely damaged and their functions are greatly disturbed. In the first group there may be extreme acidosis without intoxication; in the other the excess of acetone compounds is slight, a more definite toxic element is present from the first, and the metabolic disorder becomes complex.

Nencki and Hahn, Denys and Stube, and Pick, attributed the symptom following destruction of the liver to poisoning by ammonium carbamate, but Lieblein concludes that excess of ammonium salts does not appear until the last stages of the intoxication and that the symptoms must be attributed to the loss of other functions of the liver. The physiologic significance of carbamic acid has not been satisfactorily determined, owing to uncertainties in the available methods (McLeod, Haskins). The relation of this acid to the urinary ammonia in dogs with Eck fistulae is therefore uncertain.

In diabetic coma the abstraction of fixed alkalis is very probably an important factor, while in other conditions this influence appears less prominent, the alkalescence of the blood is not greatly reduced, and poisoning by ammonium salts, if we may accept the hypothesis of Hahn and Nencki, dominates the clinical picture. This hypothesis has not gained general acceptance, but there is no doubt of the extreme toxicity of ammonium salts as compared with that of the acetone bodies, and any one who compares the toxicity of these agents in animals can not fail to be impressed by the comparative harmlessness of the acetone

bodies and violent nervous and respiratory symptoms following injections ammonia salts. Mendel has recently urged the possible importance of the toxicity of ammonium salts in acidosis, and it is my belief that there is much clinical, pathologic and chemical evidence to support this view for certain diseases commonly regarded as forms of acid intoxication. I am not prepared to argue that the main symptoms of all these diseases are caused by ammonia poisoning, but only to state that the ammonia excretion in these conditions may and usually does have an entirely different significance from that attaching to it in diabetes and simple starvation.

Many transitional cases of acidosis undoubtedly occur, as the toxic element increases, as the scope of defective metabolism widens, and the disturbance becomes more complicated by visceral lesions, and it may be that the transitional and complex cases are so numerous as to destroy the significance of any attempt at classification. In the toxemia of pregnancy, delayed chloroform poisoning, and cyclic vomiting, there may be extensive burning of body fats, and several factors may combine to raise the ammonia. With Wolf, I have previously emphasized the importance of other changes in the nitrogen partition, especially of the amido-acid ratio, as a control of the ammonia and as indicating the influence of other disturbances of metabolism quite apart from the signs of acidosis.

While the typical cases of both classes seem to be quite distinct, the present subdivision can be suggested only as an hypothesis the validity of which must be determined by future investigations.

CONCLUSIONS.

In a field which presents the most varied and perplexing of clinical phenomena, in which pathologic anatomy offers uncertain guidance, and in which physiologic chemistry, while having to deal with many uncertain technical methods, encounters such problems as the alkalescence and ion concentration of the blood, the general significance of alkalies, the more complicated processes in many departments of metabolism, including the physiology of proteins, carbohydrates, and fats, as well as that of many inorganic principles of the body—in such a field it is not to be expected that positive answers can be given to the many questions that are being pressed. One must await further progress in collateral sciences before the significance of acidosis can be fully determined.

It is superfluous to urge the need of further work in all departments of this study. Prominent among the requirements seem to be the simplification of technical methods, the further elucidation of the alkalescence and acid-neutralizing function of the blood, the study of variations in the

alkali content of the organs, of the quantities of acetone compounds and other acids in the blood and organs of many diseases, the toxicity of acid substances and other metabolic products when combined with pre-existing lesions of the organs, and the more complete picture of nitrogenous metabolism in diseases accompanied by acidosis.

In the pursuit of these topics it should be recognized that the significance of acetonuria and of the results of many clinical methods of urine and blood analysis is not yet clear enough to render simple qualitative tests of much value; yet the importance of work of this type, if only from an educational standpoint, should not be underestimated. At the same time it must be urged that genuine progress can be attained only by the most accurate methods of fully equipped chemical and pathologic laboratories, systematically applied in cooperation with clinicians under ideal hospital facilities.

The demands of the future need not, however, obscure the importance of the results already secured. The work on acidosis is a fundamental chapter in medical science revealing in a trustworthy manner the exact nature and degree of a disturbance of metabolism which is of widespread occurrence and of prime significance in many diseases.

In the hands of the clinician it places a means of diagnosis and an insight into the nature of disease which entirely escape the reach of former methods of clinical study.

To the pathologic anatomist it has disclosed the main factors in the pathogenesis of fatty degeneration, and has given to the much-contemned autopsy finding of fatty liver a definite, new, and lively interest. Read in terms of physiologic chemistry, acute degeneration of the liver becomes a topic of first importance to the pathologist.

From the standpoint of physiologic chemistry the results seem to show that acidosis with the presence of acetone compounds usually results from abnormal metabolism of body or food fats and to a less extent of proteins, in which defective consumption of carbohydrates is an essential factor, and excessive excretion of ammonia a purely compensatory process.

In many cases, however, there is a toxic or specific element which interferes with consumption of carbohydrates, even when these are present, and leads to a rapid and dangerous burning of body fats.

In a third group of cases the excess of ammonia excretion is not a compensatory process, but the result of disturbance of urea formation, and lactic and other acids appear in excess.

The therapeutic importance of the doctrine of acid intoxication has always been recognized and sometimes, perhaps, overestimated. It seems to be time that the idea of a fatal abstraction of alkali in diabetic coma

should give place to unbiased search for other factors in this disease, some of which may be affected by treatment. It is not necessary to deny the existence of acid intoxication in order to recognize that the alkali treatment of coma has been a signal disappointment. While this treatment certainly deserves a place in the treatment of diabetes, its place must be secondary since there is no claim that it favorably influences the essential process in the disease. The remarkable antiketogenic effect of glyconic and glutaric acids suggests a new hope in the treatment of diabetic acidosis, but I learn that this hope has already met with disappointment. This result merely emphasizes the fact that diabetic coma is something more than acid intoxication.

On the other hand, it is not necessary to know all about the origin and pathogenic relations of acidosis to see the clear indications that, whenever present, it should be combated by carefully adjusted diet, by other hygienic measures, but chiefly by prophylaxis. Here lies the great practical value of the studies of acidosis, and it would be difficult to overestimate the importance to the practitioner of a detailed knowledge of all departments of the subject, and an ability to follow up its clues, especially in administering anesthetics, in the choice and preparation of subjects for operation, in the management of pregnancy and labor, in the control of gastrointestinal diseases of children, and in the general plan of dietetics in disease.

The study of acidosis shows one thing clearly: that the feeding of the healthy man, as well as the diet of the sick, can not be left to chance, guided by appetite, or ruled by tradition, but can be safely directed only according to the laws of digestion and metabolism.

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BIBLIOGRAPHY OF PART III.

- Abram: Jour. Path. and Bacteriol., 1903, iii, 430.
 Araki: Ztschr. f. physiol. Chem., 1901, xv, 335, 546.
 Baer: Arch. f. exper. Path. u. Pharmacol., 1904, li, 271
 Baer and Blum: Beitr. z. chem. Physiol. u. Path. (Hofmeister's), 1907, x, 80.
 Baginsky: Arch. f. Kinderh., 1888, ix, 1.
 Baldwin: Jour. Biol. Chem., 1905, i, 241.
 Ballin: Ann. Surg., 1903, xxxvii, 362.
 Baumstark and Mohr: Zeitschr. f. exper. Path. u. Therap., 1906, iii, 687.
 Becker: Arch. f. path. Anat., 1895, cxl, 1.
 Bevan and Favill: Jour. Am. Med. Assn., 1905, xlv, 691, 754.
 Blumenthal: Pathologie des Harnes, 1903; Berlin Urban u. Schwarzenberg.
 Boeri: Riv. clin. e. terap., 1891, xiii.
 Bönniger and Mohr: Ztschr. f. exper. Path. u. Therap., 1906, iii, 675.
 Botazzi and Orefici: Sperimentale, 1907, lv, 888.
 Brackett, Stone and Low: Boston Med. and Surg. Jour., 1904, cli, 2.
 Brewer: Ann. Surg., 1902, xxxvi, 481.
 Brugsch: Ztschr. f. exper. Path. u. Therap., 1905, i, 419.

- Brugsch and Bamberg: *Centralbl. f. Stoffwechs.- u. Verdauungsk.*, 1908, ix, 1.
 Carmichael and Beattie: *Lancet*, London, 1905, ii, 437.
 Cathcart: *Jour. Physiol.*, 1907, xxxv, 500.
 Cassaet: *Arch. gén. de méd.*, 1901, i, 257, 431.
 Cohnstein and Michaelis: *Arch. f. d. ges. Physiol.*, 1897, lxv, 475; 1898, lxix, 76.
 Comby: *Bull. de pédiat. de Paris*, 1905, vii, 53.
 Couvelaire: *Ann. de Gynéc. et d'Obst.*, 1899, ii, 417.
 De Boeck and Slosse: *Bull. Soc. de méd. ment. de Belgique*, 1891, No. 62, 301.
 Denys and Stube: *Centralbl. f. allg. Path.*, 1893, iv, 102.
 Dreyfuss: *Biochem. Ztschr.*, 1908, vii, 493.
 Ebstein: *Deutsch. Arch. f. klin. Med.*, 1882, xxx, 1.
 Edgar: *New York Med. Jour.*, 1906, lxxxiii, 897, 957.
 Edsall: *Am. Jour. Med. Sc.*, 1903, cxxv, 629.
 Engel: *Ztschr. f. klin. Med.*, 1892, xx, 514.
 Ewing: *Tr. Philadelphia Path. Soc.*, 1905, viii, 56; *New York Med. Rec.*, 1907, lxxi, 537; *Am. Jour. Obst.*, 1905, li, 145.
 Ewing and Wolf: *Am. Jour. Obst.*, 1907, lv, 289.
 Fenwick: *Disorders of Digestion in Infancy and Childhood*, London, 1897.
 H. K. Lewis, p. 233.
 Folin: *Amer. Jour. Physiol.*, 1905, xiii, 117.
 Fraenkel: *Deutsch. med. Wchnschr.*, 1901, 196.
 Freund, E. and O.: *Wien. klin. Rundschau*, 1901, 69, 91.
 Gee: *St. Barth. Hosp. Rep.*, 1882, xviii, 1.
 Geelmuyden: *Ztschr. f. physiol. Chem.*, 1897, xxiii, 431; *Scand. Arch. f. Physiol.*, 1901, xi, 97.
 Gerhardt and Schlesinger: *Arch. f. exper. Path. u. Pharmakol.*, 1899, xlii, 83.
 Griffith: *Am. Jour. Med. Sc.*, 1900, cxx, 553.
 Guthrie: *Lancet*, London, 1894, i, 193, 257; 1903, ii, 10.
 Hahn, Massin, Nencki and Pawlow: *Arch. f. exper. Path. u. Pharmakol.*, 1893, xxxii, 161, 185.
 Hirschfeld: *Ztschr. f. klin. Med.*, 1895, xxviii, 176.
 Hirschler: *Ztschr. f. physiol. Chem.*, 1886, x, 315.
 Holt: *Diseases of Infancy and Childhood*, New York, 1902, D. Appleton & Co., 287.
 von Jaksch: *Ueber Acetonurie und Diaceturie*, Berlin, 1885; *Ztschr. f. klin. Med.*, 1886, x, 362; *Deutsche med. Wchnschr.*, 1893, xix, 10.
 von Jauregg: *Wien. klin. Wchnschr.*, 1896, ix, 165.
 Kelly: *Ann. Surg.*, 1905, xli, 161.
 Klemperer: *Berl. klin. Wchnschr.*, 1889, xxvi, 869.
 Knapp: *Centralbl. f. Gynäk.*, 1897, xxi, 417.
 Kraus: *Ergebn. d. allg. Path. u. path. Anat.* (Lubarsch and Osterag), 1895, ii, 618.
 Külz: *Ztschr. f. Biol.*, 1887, xxiii, 329.
 Lambert and Wolf: Personal communication.
 Langmead: *Brit. Med. Jour.*, 1905, i, 350.
 Leyden: *Ztschr. f. klin. Med.*, 1882, iv, 605.
 Litten: *Ztschr. f. klin. Med.*, 1884, vii, suppl., 81.
 Lieblein: *Arch. f. exper. Path. u. Pharmakol.*, 1894, xxxiii, 318.
 Loewy and Münzer: *Deutsch. Arch. f. klin. Med.*, 1901, lxxxii, 174.
 Lorenz: *Ztschr. f. klin. Med.*, 1891, xix, 19.
 Lüthje: *Centralbl. f. inn. Med.*, 1899, xx, 969.
 Macleod and Haskins: *Am. Jour. Physiol.*, 1905, xii, 444.
 Marcy: *Internat. Clin.*, 1899, Series 9, iii, 127.
 Marfan: *Bull. Soc. de Pédiat. de Paris*, 1905, vii, 41.
 Marum: *Beitr. z. chem. Physiol.*, 1907, x, 105.
 Mavrakis: *Arch. f. Anat. u. Physiol., Phys. Aht.*, 1904, i, 94.

- Mendel: Tr. Assn. Am. Phys., 1907, xxii, 265.
 Mercier and Menu: Soc. d'obst. de Paris, 1899, ii, 224.
 Meyer: Arch. f. exper. Path. u. Pharmakol., 1881, xiv, 313.
 Meyer, L. (cited by von Noorden): Loc. cit., ii, 49, 50.
 Minkowski: Ergebn d. allg. Path. u. path. Anat., 1895, ii, 721.
 Mohr: Samml. klin. abhandl. (von Noorden), 1904, iv.
 Morpurgo: Arch. ital. di Biol., 1889, xii, 333.
 Müller, F.: Ztschr. f. klin. Med., 1889, xvi, 496.
 Müller, F., and Senator: Arch. f. path. Anat., 1893, cxxxi, suppl.
 Munzer: Deutsch. Arch. f. klin. Med., 1894, lii, 199.
 Naunyn: Specielle Pathologie und Therapie, Nothnagel; vi, Par 1, 1900, Vienna, A. Holder.
 Nebelthau: Centralbl. f. inn. Med., 1897, xviii, 977.
 Nencki and Hahn: Arch. f. exper. Path. u. Pharmakol., 1893, xxxii, 161, 185.
 Nicolaides: Arch. f. Physiol. (Engelmann), 1899, 518.
 von Noorden: Pathologie des Stoffwechsels, Berlin, 1893, A. Hirschwald;
 Metabolism and Practical Medicine, 1907, ii, 35.
 Pawinski: Berl. klin. Wehnschr., 1888, xxv, 1004.
 Pepper: Cyclopedia of Diseases of Children (Keating), iii, 22; also supplement, 631.
 Pfaundler: Munchen. med. Wehnschr., 1902, p. 1211.
 Pick: Arch. f. exper. Path. u. Pharmakol., 1893, xxxii, 382.
 Rachford: Arch. Pediat., 1897, xiv, 561, 661, 742; 1898, xv, 605.
 Remond: Arch. gén. de méd., 1889, clxix, 38.
 Richards and Howland: Arch. Pediat., 1907, xxiv, 401.
 Riess: Ztschr. f. klin. Med., 1884, vii, Supplement, 34.
 Rosenfeld: Centralbl. f. inn. Med., 1895, xvi, 1233.
 Satta: Beitr. z. chem. Physiol., 1905, vi, 1.
 Saveliëff: Berl. klin. Wehnschr., 1894, xxxi, 754.
 Saxl: Beitr. z. chem. Physiol. u. Path. (Hofmeister's), 1907, x, 447.
 Schenk: Ztschr. f. Heilk., 1898, xix, 93.
 Scholten: Beitr. z. Geburtsch. u. Gynäk. (Hegar's), 1900, iii, 3.
 Schöndorff: Arch. f. d. ges. Physiol., 1893, liv, 420.
 Schrack: Jahrb. d. Kinderh., 1899, xxix, 411.
 Schulz: Arch. f. d. ges. Physiol., 1899, lxxvi, 379.
 Schwarz: Deutsch. Arch. f. klin. Med., 1903, lxxvi, 233.
 Shaw and Tribe: Brit. Med. Jour., 1905, i, 347.
 Stiles and McDonald: Scot. Med. and Surg. Jour., 1904, xv, 97.
 Stolz: Arch. f. Gynäk., 1902, lxxv, 531.
 Stone: Am. Gynec., 1903, iii, 518; New York Med. Rec., 1905, lxxviii, 295.
 Symes: Dublin Jour. Med. Sci., 1897, civ, 112.
 Tuzek: Arch. f. Psychiat. u. Nervenkr., 1884, xv, 784.
 Valagusa: Policlinico (See Med.), 1902, iv, 555.
 Vergely: Rev. mens. d. mal. de l'enf., 1898, xvi, 1.
 Vicarelli: Prag. med. Wehnschr., 1893, xviii, 403, 428.
 Waldvogel: Die Acetonkörper, Stuttgart, 1903, F. Enke., p. 117.
 Walko: Ztschr. f. Heilk., 1901, xxxii, 145.
 Williams: Johns Hopkins Hosp. Bull., 1906, xvii, 71.
 Zangemeister: Ztschr. f. Geburtsch. u. Gynäk., 1901, v, 310.
 Zweifel: Arch. f. Gynäk., 1904, lxxii, 1; lxxvi, 536.

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AMERICAN MEDICAL ASSOCIATION, ONE HUNDRED AND THREE DEARBORN AVENUE.
 CHICAGO.

Die gegenseitige Ausflockung von Kolloiden.

Von

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In einer frühern Mitteilung²⁾ ist gezeigt worden, dass bei der gegenseitigen Ausflockung von Farbstoffen sowohl die Vollständigkeit der Ausflockung, als auch die Ausdehnung der Ausflockungszone von der mehr oder minder kolloidalen Natur der verwendeten Farbstoffe abhängig ist. So zeigen Eosin und Bismarckbraun, die beide leicht durch Pergamentpapier diffundieren, bei keiner erreichbaren Konzentration vollständige Ausflockung beider Farbstoffe, dagegen eine unvollkommene Ausflockung innerhalb eines weiten Konzentrationsbereichs. Kongorot und Nachtblau anderseits sind beide hochkolloidal und zeigen nur dann eine Ausflockung, wenn sie in gewissen, sehr bestimmten Verhältnissen zugegen sind; aber bei diesen Verdünnungen ist die Ausflockung für beide Farbstoffe vollständig, so dass die überstehende Flüssigkeit farblos wird. Diese Regel, welche wir die „Regel der kolloidalen Ausflockung“ zu nennen vorschlagen, wurde für eine lange Reihe von untersuchten Farbstoffen als gültig befunden, und es wurde bemerkt, dass sie allgemeiner Anwendbarkeit fähig sein könnte, oder, mit andern Worten, dass sie für andere kolloidale Lösungen ebensogut Gültigkeit besitzen könnte, wie für die Farbstoffe. Einige vorläufige Versuche mit Bakterien, welche sich der Regel anzupassen schienen, sind damals ebenfalls angegeben worden, und die vorliegende Untersuchung sollte dazu dienen, die Frage weiter zu beleuchten.

Die zu den Versuchen verwendeten Kolloide waren möglichst verschiedener Natur.

¹⁾ Aus dem Englischen übersetzt von W. Neumann.

²⁾ Zeitschr. f. physik. Chemie **60**, 409 (1907).

Anorganisch	Positiv	Ferrihydroxyd
	„	Aluminiumhydroxyd
	Negativ	Kolloidales Platin
	„	Arsensulfid
	„	Russ
Organisch (alles negative Kolloide)	Nicht stickstoffhaltig	Mastix
	„ „	Tannin
	„ „	Stärke
	Stickstoffhaltig	Serum
	„	Hämoglobin
	„	Lecithin
	„	Gelatine
	Mikroskopische Suspensionen	Bakterien
	„ „	Agglutininbakterien

Als Ausflockungsmittel für diese Reihe von Kolloiden wählten wir eine Anzahl von sauren und basischen Farbstoffen (von Grüber für histologische Färbzwecke), die in Tabelle 1 angegeben sind. Ihr relativer kolloidaler Charakter wurde mit Hilfe der Dialyse durch gewöhnliches Pergamentpapier geprüft. Die hauptsächlichsten benutzten basischen Farbstoffe dialysierten in der Reihenfolge: Methylenblau, Neutralrot, Nilblau, Janusgrün, Nachtblau. Nach der Regel der kolloidalen Ausflockung sollte die Ausflockung durch diese eine negative Ladung tragenden Kolloide im Falle von Janusgrün und Nachtblau vollständig sein, und zwar mit einer engen Ausflockungszone. Mit Methylenblau und Neutralrot dagegen sollte die Ausflockung unvollständig und die Ausflockungszone weit sein, oder es sollte möglicherweise gar keine Ausfällung eintreten. Nilblau sollte eine Mittelstellung einnehmen. Ein flüchtiger Blick auf die Tabellen lehrt, dass diese Regel in fast allen Fällen zutrifft. Das Verhalten von Neutralrot ist etwas merkwürdig. Es scheint durch gewöhnliches Pergamentpapier etwas leichter zu dialysieren, als Eosin oder Alizarinrot, aber in Schleicher und Schülls Dialysierbechern von stärkerm Pergament untersucht, dialysiert Neutralrot verhältnismässig langsam, so dass wir es in unserer letzten Abhandlung zu den mässig kolloidalen Farbstoffen zählten. In seinem Ausflockungsverhalten erscheint es ausgesprochen kolloidaler als Methylenblau, aber weniger kolloidal als Nilblau.

Die benutzten sauren Farbstoffe dialysierten in der Reihenfolge: Alizarinrot, Eosin, Biebricher Scharlach, Nigrosin, Kongorot, und das basische Ferri- oder Aluminiumhydroxyd sollte daher durch Nigrosin oder Kongorot mit einer engen Ausflockungszone vollständiger ausgeflockt werden, als durch Alizarinrot oder Eosin, welche letztere eine

weite Zone unvollkommener Ausflockung besitzen sollten. Biebricher Scharlach müsste eine Mittelstellung einnehmen.

Da das hier hauptsächlich Hervorzuhebende die relative Breite der Ausflockungszonen ist, haben wir es für ratsam gehalten, die Resultate von jedem Versuche ausführlich anzugeben, so dass ein Blick auf die Tabellen zur Erkennung der Unterschiede der Wirkungsweise kolloidalerer und weniger kolloidaler Farbstoffe genügt. Die Tabellen hätten erheblich gekürzt werden können, aber dann würde jede von ihnen einiges Studium zur Bewertung der Unterschiede in den Ausflockungszonen erfordern. Jeder Versuch ist wenigstens einmal, und viele von ihnen sind häufig wiederholt worden, ohne dass irgend welche bemerkenswerte Abweichungen aufgetreten wären.

Tabelle 1.

Dialyse.

Bei Zimmertemperatur.

	15 Min.	30 Min.	45 Min.	1 Std.	1½ Std.	2 Std.	3½ Std.	5 Std.	24 Std.	37° 5 Std. lang
Kongorot	—	—	—	—	—	—	—	—	—	—
Trypanrot	—	—	—	—	—	—	—	—	—	Spur
Nachtblau	+	—	—	—	—	—	—	—	—	+
Janusgrün	+	—	—	—	—	—	—	—	+	++
Nigrosin	—	—	—	—	—	—	—	—	+	++
Nilblau	+	—	—	—	—	Spur	+	++	+++	—
Biebricher Scharlach	—	—	—	Spur	Spur	Spur	+	++	+++	—
Eosin	—	—	geringe Spur	Spur	Spur	Spur	+	++	+++	—
Alizarinrot	—	—	geringe Spur	Spur	Spur	Spur	+	++	+++	—
Neutralrot	+	—	geringe Spur	Spur	+	++	++	++	+++	—
Methylenblau	+	Spur	deutl. Spur	++	++	++	++	++	+++	—

Es mögen jetzt die einzelnen Tabellen erläutert werden. Die Tabellen 2 und 3 geben, als Auswahl unter einer sehr grossen Anzahl von Versuchen, Beispiele von der Art und Weise, in welcher die Wirkung der Farbstoffe aufeinander zur vorläufigen Feststellung untersucht wurde, wobei gewisse, in den frühern Versuchen noch nicht benutzte Farbstoffe, z. B. Trypanrot, Janusgrün, Nigrosin, Biebricher Scharlach Anwendung fanden. Die Ausflockung zeigt nach beiden Tabellen Anpassung an die oben ausgesprochene Regel der kolloidalen Ausflockung; Trypanrot, das hochkolloidal ist, gibt mit Janusgrün und Nachtblau sehr enge Zonen, aber sehr weite mit Neutralrot und Methylenblau, obgleich das dazwischenliegende Nilblau eine engere Zone zeigt, als theoretisch zu erwarten wäre.

Tabelle 2.

Hochkolloidaler saurer Farbstoff mit verschiedenen basischen Farbstoffen.
Trypanrot: $\frac{1}{400}$ % endgültige Verdünnung.

Nr.	$Fe(OH)_3$ und basische Farb- stoffe. Endgültige Verdünnung %	Basisches Hydroxyd $Fe(OH)_3$	Hochkolloidale Farb- stoffe		Mässig kolloidal	Wenig kolloidale Farbstoffe	
			Nachtblau	Janusgrün	Nilblau	Neutralrot	Methylen- blau
1	$\frac{1}{30}$	—	—	—	—	—	+++
2	$\frac{1}{40}$	—	—	—	—	—	+++
3	$\frac{1}{60}$	+	—	—	—	—	+++
4	$\frac{1}{80}$	+++	—	—	—	—	+++
5	$\frac{1}{100}$	+++	—	—	—	+	+++
6	$\frac{1}{120}$	++	—	+	—	+	+++
7	$\frac{1}{140}$	—	—	+++	—	+++	+++
8	$\frac{1}{160}$	—	+++	—	—	+++	+++
9	$\frac{1}{180}$	—	—	—	—	+++	+++
10	$\frac{1}{200}$	—	—	—	—	+++	+++
11	$\frac{1}{240}$	—	—	—	+++	+++	+++
12	$\frac{1}{280}$	—	—	—	—	+++	+++
13	$\frac{1}{320}$	—	—	—	—	+++	+++
14	$\frac{1}{360}$	—	—	—	—	+++	—
15	$\frac{1}{400}$	—	—	—	—	+	—
16	$\frac{1}{480} - \frac{1}{\infty}$	—	—	—	—	—	—

Tabelle 3.

Hochkolloidaler basischer Farbstoff mit verschiedenen sauren Farbstoffen.
Janusgrün, endgültige Verdünnung: $\frac{1}{200}$ %.

Nr.	Saure Farb- stoffe. Endgültige Verdünnung %	Hochkolloidale Farb- stoffe		Mässig kolloidal	Wenig kolloidale Farb- stoffe	
		Kongorot	Nigrosin	Biebricher Scharlach	Eosin	Alizarin- rot
1	$\frac{1}{30}$	—	—	—	?	—
2	$\frac{1}{40}$	—	—	—	+++	—
3	$\frac{1}{60}$	—	—	—	+++	—
4	$\frac{1}{80}$	—	—	—	+++	+++
5	$\frac{1}{100}$	—	—	—	+++	+++
6	$\frac{1}{120}$	—	—	—	+++	+++
7	$\frac{1}{140}$	—	—	—	+++	+++
8	$\frac{1}{160}$	—	—	—	+++	+++
9	$\frac{1}{180}$	—	—	—	+++	+++
10	$\frac{1}{200}$	—	—	+	+++	+++
11	$\frac{1}{240}$	—	+++	+++	+++	+++
12	$\frac{1}{280}$	—	++	+++	+++	+++
13	$\frac{1}{320}$	—	+	+	+++	+++
14	$\frac{1}{360}$	—	—	+	+++	+++
15	$\frac{1}{400}$	+	—	+	+++	+++
16	$\frac{1}{480}$	+++	—	—	—	+++
17	$\frac{1}{560}$	—	—	—	—	+++
18	$\frac{1}{640}$	—	—	—	—	+++
19	$\frac{1}{720}$	—	—	—	—	—
20	$\frac{1}{800} - \frac{1}{\infty}$	—	—	—	—	—

Des weitem zeigt das hochkolloidale basische Janusgrün enge Flockungszonen mit Kongorot und Nigrosin eine etwas weitere Zone mit Biebricher Scharlach, während mit Eosin und Alizarinrot die Zonen noch viel weiter sind. Dieses Verhalten entspricht in jeder Hinsicht der angeführten Regel.

Die Ausflockung mit Methylenblau und Neutralrot in Tabelle 2 und mit Eosin und Alizarinrot in Tabelle 3 ist als +++ bezeichnet worden, da eine beträchtliche Ausfällung stattgefunden hat, obgleich in keinem dieser Fälle die überstehende Flüssigkeit farblos erschien, wie dies bei der Optimalverdünnung für zwei hochkolloidale Farbstoffe immer zutrifft.

Tabelle 4.

Kolloidale basische Hydroxyde mit sauren Farbstoffen.

Nr.	Saure Farbstoffe. Endgültige Verdünnungen %	Ferrihydroxyd $\frac{1}{500}$ -original					Aluminiumhydroxyd $\frac{1}{500}$ -original				
		Hochkolloidal		Mässig kolloidal	Wenig kolloidal		Hochkolloidal		Mässig kolloidal	Wenig kolloidal	
		Kongo-rot	Nigrosin	Biebricher Scharlach	Eosin	Alizarin-rot	Kongo-rot	Nigrosin	Biebricher Scharlach	Eosin	Alizarin-rot
1	$\frac{1}{50}$	—	—	+++	++	—	—	—	+++	?	—
2	$\frac{1}{40}$	—	—	+++	++	—	—	—	+++	++	—
3	$\frac{1}{30}$	—	—	+++	++	—	—	—	+++	++	—
4	$\frac{1}{20}$	—	—	+++	++	—	—	+++	+++	++	—
5	$\frac{1}{100}$	—	—	+++	++	—	—	+++	+++	++	+
6	$\frac{1}{120}$	—	—	+++	++	—	++	+++	+++	++	+
7	$\frac{1}{140}$	—	—	+++	++	—	+++	—	+++	++	+++
8	$\frac{1}{160}$	—	—	+++	++	—	+++	—	+++	—	+++
9	$\frac{1}{180}$	—	+++	+++	++	—	—	—	+++	—	+++
10	$\frac{1}{200}$	—	+++	+++	++	—	—	—	+++	—	+++
11	$\frac{1}{240}$	—	+++	+++	++	—	—	—	++	—	+
12	$\frac{1}{280}$	+++	+++	+++	++	—	—	—	—	—	+
13	$\frac{1}{320}$	+++	+	+++	++	—	—	—	—	—	—
14	$\frac{1}{360}$	+++	+	+++	++	+	—	—	—	—	—
15	$\frac{1}{400}$	+++	—	+++	+	+	—	—	—	—	—
16	$\frac{1}{480}$	++	—	+	—	+++	—	—	—	—	—
17	$\frac{1}{560}$	+	—	+	—	+++	—	—	—	—	—
18	$\frac{1}{640}$	—	—	—	—	+++	—	—	—	—	—
19	$\frac{1}{720}$	—	—	—	—	+++	—	—	—	—	—
20	$\frac{1}{800}$	—	—	—	—	++	—	—	—	—	—
21	$\frac{1}{960}$	—	—	—	—	—	—	—	—	—	—
22	$\frac{1}{1120} - \frac{1}{\infty}$	—	—	—	—	—	—	—	—	—	—

Tabelle 4 ist eine Wiederholung von Tabelle 3, mit dem Unterschied, dass Ferri- und Aluminiumhydroxyd an Stelle von Janusgrün verwendet werden. Die Hydroxyde waren durch Fällung des Chlorids mit Kalilauge, Zusatz eines Überschusses des Chlorids und lang fortgesetzte Dialyse hergestellt worden. Das Ferrihydroxyd wurde uns von Herrn Dr. Field vom „New-York Health Department“ freundlichst

zur Verfügung gestellt. Die ursprüngliche Aluminiumhydroxydlösung war stärker, als die Lösung von Ferrihydroxyd, so dass für erstere Lösung die Ausflockungszone in der Tabelle weiter oben liegt, aber im Prinzip sind beide Fälle gleich, und die Regel der kolloidalen Ausflockung wird in allen Fällen befolgt, ausgenommen den Fall von Alizarinrot. Diese sehr ausgesprochene Ausnahme ist im Hinblick auf die Tatsache von Interesse, dass die Alizarine mit den Metallhydroxyden besonders beständige Lacke bilden. Die Stabilität dieser Lacke soll eine Funktion der OH-Gruppierung des Alizarinradikals sein [Georgevicz¹⁾, Nietzki²⁾ und andere Autoritäten].

In diesem Ausnahmefall scheint daher die Konstitution des Farbstoffs der Hauptfaktor für die Vollständigkeit der Ausflockung und die Ausdehnung der Vorzone zu sein.

Tabelle 5.

Organische Kolloide. Kolloidales Platin und Arsensulfid.

Nr.	$Fe(OH)_3$ und basische Farbstoffe. Endgültige Verdünnungen %	Kolloidales Platin					Arsensulfid						
		$Fe(OH)_3$	Nacht- blau	Janus- grün	Nilblau	Neutral- rot	Methylen- blau	$Fe(OH)_3$	Nacht- blau	Janus- grün	Nilblau	Neutral- rot	Methylen- blau
1	$\frac{1}{30} - \frac{1}{300}$	—	—	—	—	—	+++	—	—	—	—	—	—
2	$\frac{1}{340}$	+++	—	—	—	—	+++	—	—	—	—	—	—
3	$\frac{1}{380}$	+++	—	—	—	—	+++	+++	—	—	—	—	—
4	$\frac{1}{390}$	+++	—	—	—	—	+++	+++	—	—	—	—	—
5	$\frac{1}{390}$	+++	—	—	—	—	+++	+++	—	+++	—	—	—
6	$\frac{1}{400}$	+++	—	—	—	—	+++	+++	—	+++	—	—	—
7	$\frac{1}{480}$	+++	—	—	—	—	+++	+++	+++	—	—	—	—
8	$\frac{1}{500}$	+++	—	—	—	—	+++	+++	+++	—	+++	—	+++
9	$\frac{1}{640}$	+++	—	—	—	+++	+++	+++	—	—	+	—	+++
10	$\frac{1}{720}$	+++	—	—	—	+++	+++	+++	—	—	+++	+++	+++
11	$\frac{1}{800}$	+++	—	—	—	+++	+++	+++	—	—	—	+++	—
12	$\frac{1}{900}$	+++	—	—	—	+++	+++	+++	—	—	—	+++	—
13	$\frac{1}{1120}$	—	+++	—	—	+++	+++	+++	—	—	—	—	—
14	$\frac{1}{1380}$	—	+++	—	—	+++	+++	+++	—	—	—	—	—
15	$\frac{1}{1600}$	—	+++	+++	+++	+++	+	—	—	—	—	—	—
16	$\frac{1}{1920}$	—	—	+++	+++	+++	—	—	—	—	—	—	—
17	$\frac{1}{3200}$	—	—	—	+++	+	—	—	—	—	—	—	—
18	$\frac{1}{6400} - \frac{1}{\infty}$	—	—	—	—	—	—	—	—	—	—	—	—

Tabelle 5 zeigt die Resultate mit den anorganischen Kolloiden Platin und Arsensulfid. Das kolloidale Platin wurde nach der Bredig'schen Methode durch Erzeugung eines Lichtbogens unter Wasser hergestellt, und sein Verhalten bei der Ausflockung scheint der oben erwähnten Regel zu entsprechen.

¹⁾ Chemical Technology of the textile fibres, übersetzt von Salter, 1902.

²⁾ Organische Farbstoffe. 5. Aufl. 1906.

Zur Herstellung des Arsensulfids wurde arsenige Säure in destilliertem Wasser gekocht und die Lösung in Schwefelwasserstoffwasser gegossen, worauf der Überschuss an H_2S durch einen Wasserstoffstrom vertrieben wurde. Arsensulfid scheint eine Ausnahme von der Regel der kolloidalen Ausflockung zu bilden, da Neutralrot und Methylenblau ganz ebenso enge Zonen zeigen, wie Nachtblau und Janusgrün. Es ist möglich, dass Spuren von in der Lösung verbliebenem H_2S zu diesem abnormen Verhalten beigetragen haben, da H_2S -Wasser selbst eine geringe Ausflockungswirkung auf Methylenblau ausübt. Der Versuch ist deshalb nicht über jeden Verdacht erhaben.

Tabelle 6.

Nichtstickstoffhaltige organische Kolloide, Mastix und Tannin.

Nr.	$Fe(OH)_3$ und basische Farbstoffe. Endgültige Verdünnungen	Mastix $\frac{1}{30}$ Orig.					Tannin $\frac{1}{10}$ %						
		$Fe(OH)_3$	Nacht- blau	Janus- grün	Nilblau	Neutral- rot	Methylen- blau	$Fe(OH)_3$	Nacht- blau	Janus- grün	Nilblau	Neutral- rot	Methylen- blau
1	1	—	—	—	—	—	2	+++	—	—	—	—	—
2	$\frac{1}{20}$	—	—	—	—	—	+++	+++	—	—	—	—	—
3	$\frac{1}{40}$	—	—	—	—	—	+++	+++	—	—	—	—	—
4	$\frac{1}{60}$	—	—	—	—	—	+++	+++	—	—	—	—	—
5	$\frac{1}{80}$	—	—	—	—	—	+++	++	—	—	—	—	—
6	$\frac{1}{100}$	—	—	—	—	—	+++	+	—	—	—	—	—
7	$\frac{1}{120}$	—	—	—	—	—	+++	+	—	—	—	—	—
8	$\frac{1}{140}$	—	—	—	—	—	+++	+	—	—	—	—	—
9	$\frac{1}{160}$	—	—	—	—	—	+++	+	—	—	—	—	—
10	$\frac{1}{180}$	—	—	—	—	—	+++	+	—	—	—	—	—
11	$\frac{1}{200}$	—	—	—	—	—	++	+	—	—	—	—	—
12	$\frac{1}{240}$	—	—	—	—	—	+	Spur	—	—	—	—	—
13	$\frac{1}{280}$	—	—	—	—	—	—	Spur	—	—	—	—	—
14	$\frac{1}{320}$	—	—	—	—	+	—	Spur	—	—	—	—	—
15	$\frac{1}{360}$	—	—	—	—	++	—	Spur	—	—	—	—	—
16	$\frac{1}{400}$	—	—	—	—	+++	—	—	—	—	—	—	—
17	$\frac{1}{480}$	+	—	—	—	+++	—	—	—	—	—	—	—
18	$\frac{1}{560}$	+++	—	—	—	+++	—	—	—	+++	—	—	—
19	$\frac{1}{640}$	+++	—	—	—	+++	—	—	+++	+++	—	—	—
20	$\frac{1}{720}$	+++	—	—	++	+++	—	—	+++	+++	—	—	—
21	$\frac{1}{800}$	+++	—	—	++	+++	—	—	—	+++	+++	—	—
22	$\frac{1}{960}$	+++	—	—	—	++	—	—	Spur	+++	+++	—	—
23	$\frac{1}{1120}$	+++	—	—	—	+	—	—	+++	+++	—	—	—
24	$\frac{1}{1280}$	+++	++	++	+++	—	—	—	+++	+++	—	—	—
25	$\frac{1}{1600}$	+	+++	+++	+++	—	—	—	+++	+++	—	—	—
26	$\frac{1}{1920}$	+	+	+	++	—	—	—	++	+++	—	—	—
26	$\frac{1}{2200}$	—	—	—	—	—	—	—	—	—	—	—	—

Tabelle 6. — Nichtstickstoffhaltige Kolloide. Mastixsuspension und Tanninlösung.

Zur Bereitung der Mastixlösung wurden 10 ccm einer gesättigten alkoholischen Lösung langsam zu 90 ccm Wasser gefügt, und diese Lö-

sung wurde nach Bechholds Vorgang „orig.“ genannt. Zu den Versuchen wurde die „Original“-Suspension filtriert und auf das Dreissigfache verdünnt. Mastix scheint der Regel der kolloidalen Ausflockung zu folgen, da die Zonen für Nachtblau und Janusgrün besonders eng, aber für Neutralrot und Methylenblau weit sind. Im Falle des Nilblaus tritt eine Unregelmässigkeit in der Ausflockungszone auf: ein Beispiel für die Bechholdschen „unregelmässigen Reihen“. Derartige Unregelmässigkeiten kommen auch in manchen der übrigen Tabellen vor, sollen aber in dieser Abhandlung nicht weiter erörtert werden.

Mit $\frac{1}{10}\%$ igem Tannin finden wir für Nachtblau und Janusgrün ziemlich breite Zonen, während die drei weniger kolloidalen Farbstoffe überhaupt keine Ausflockung hervorrufen. Ferrihydroxyd zeigt eine breite Zone, und in andern Versuchen mit 1- und 3%iger Tanninlösung entstand in jedem Röhrchen eine deutliche Fällung, zweifellos infolge der Bildung eines Eisentannats, das bei allen Verdünnungen unlöslich ist. Infolgedessen ist die Regel der kolloidalen Ausflockung hier nicht anwendbar.

Tabelle 7.

Stickstoffhaltige Kolloide.

Lezithin und Hämoglobin.

Nr.	$Fe(OH)_3$ und basische Farbstoffe. Endgültige Verdünnungen %	Lezithin						Hämoglobin					
		$Fe(OH)_3$	Nacht- blau	Janus- grün	Nilblau	Neutral- rot	Methylen- blau	$Fe(OH)_3$	Nacht- blau	Janus- grün	Nilblau	Neutral- rot	Methylen- blau
1	$\frac{1}{20}$	—	—	—	—	—	—	+	—	—	++	+	Spur
2	$\frac{1}{40}$	—	—	—	—	—	—	+++	—	—	++	+	Spur
3	$\frac{1}{60}$	—	—	—	—	—	—	++	—	—	++	+	Spur
4	$\frac{1}{80}$	—	—	—	—	—	—	—	—	—	++	+	Spur
5	$\frac{1}{100}$	—	—	—	—	+	—	—	+	—	++	+	Spur
6	$\frac{1}{120}$	+++	—	—	—	+	—	—	+++	—	++	+	—
7	$\frac{1}{140}$	+++	—	—	—	+	—	—	+++	—	++	+	—
8	$\frac{1}{160}$	+++	—	—	—	++	—	—	+++	+++	++	—	Spur
9	$\frac{1}{180}$	+++	—	—	—	++	—	—	+++	+++	++	—	Spur
10	$\frac{1}{200}$	—	—	—	—	++	—	—	+++	+++	++	—	Spur
11	$\frac{1}{240}$	—	—	+++	—	++	—	—	+++	+++	++	—	Spur
12	$\frac{1}{280}$	—	—	Spur	—	++	—	—	—	+++	++	—	Spur
13	$\frac{1}{320}$	—	—	—	++	++	—	—	—	+	—	—	Spur
14	$\frac{1}{360}$	—	++	—	—	++	—	—	—	+	—	—	Spur
15	$\frac{1}{400}$	—	++	—	++	+	—	—	—	—	—	—	Spur
16	$\frac{1}{480}$	—	—	—	Spur	—	—	—	—	—	—	—	Spur
17	$\frac{1}{560}$	—	—	—	Spur	—	—	—	—	—	—	—	Spur
18	$\frac{1}{640}$	—	—	—	—	—	—	—	—	—	—	—	Spur
19	$\frac{1}{720}$	—	—	—	—	—	—	—	—	—	—	—	—
20	$\frac{1}{800} - \frac{1}{\infty}$	—	—	—	—	—	—	—	—	—	—	—	—

Tabelle 8.
Stickstoffhaltige Kolloide.
Serum erhitzt und nicht erhitzt.

Nr.	$Fe(OH)_3$ und basische Farbstoffe. Endgültige Ver- dünnungen %	$Fe(OH)_3$		Nachtblau		Janusgrün		Nilblau		Neutralrot		Methylenblau	
		Serum		Serum		Serum		Serum		Serum		Serum	
		Normal	Erhitzt	Normal	Erhitzt	Normal	Erhitzt	Normal	Erhitzt	Normal	Erhitzt	Normal	Erhitzt
1	1/20	—	—	—	—	—	—	++	—	+	—	Spur	++
2	1/40	—	—	—	—	++	—	++	—	+	+++	Spur	++
3	1/60	+	—	—	—	+++	+	++	++	+	+++	Spur	++
4	1/80	+++	—	+	—	+++	+++	++	+++	+	+++	Spur	++
5	1/100	+++	+++	+++	+++	+++	+++	++	+++	+	+++	Spur	++
6	1/120	+++	+++	+++	+++	+++	+	++	+++	+	+++	—	++
7	1/140	+++	+++	+++	+++	+++	—	++	+++	+	++	—	—
8	1/160	+++	+++	+++	—	+++	—	++	—	+	++	—	—
9	1/180	+++	+++	+++	—	+++	—	++	—	+	—	—	—
10	1/200	—	—	+++	—	+++	—	++	—	—	+	—	—
11	1/240	—	—	+	—	+++	—	++	—	—	+	—	—
12	1/280	—	—	—	—	+++	—	++	—	—	+	—	—
13	1/320	—	—	—	—	+++	—	++	—	—	+	—	—
14	1/360	—	—	—	—	—	—	++	—	—	+	—	—
15	1/400	—	—	—	—	—	—	+	—	—	—	—	—
16	1/480	—	—	—	—	—	—	—	—	—	—	—	—
17	1/560	—	—	—	—	—	—	—	—	—	—	—	—
18	1/640	—	—	—	—	—	—	—	—	—	—	—	—
19	1/720	—	—	—	—	—	—	—	—	—	—	—	—
20	1/800 — 1/∞	—	—	—	—	—	—	—	—	—	—	—	—

Tabellen 7 und 8. — Stickstoffhaltige Kolloide, Lecithin, Hämoglobin und Blutserum.

Lecithin. 3 ccm einer gesättigten alkoholischen Lösung von Lecithin wurden zu 100 ccm Wasser gefügt, die Suspension wurde darauf zur Vertreibung des Alkohols eine Stunde lang auf dem Wasserbade erhitzt und mit weitem 100 ccm Wasser verdünnt. Abgesehen davon, dass die Ausflockung mit Nachtblau etwas unvollkommen ist, wird die Regel gut befolgt.

Hämoglobin. Die roten Blutkörperchen von 20 ccm defibriniertem Schafblut wurden viermal mit 7%iger Rohrzuckerlösung gewaschen, um das Serum zu entfernen, und dann in 100 ccm Wasser der Hämolyse unterworfen. Die entstehende Lösung wurde filtriert und auf das Fünffache verdünnt, da sich diese Verdünnung als für die Versuche passend erwies.

Die Regel der kolloidalen Ausflockung gilt auch für das Hämoglobin, obgleich die Ausflockungszone mit den hochkolloidalen Farbstoffen von beträchtlicher Ausdehnung ist. Die wenig kolloidalen Farbstoffe reagieren wenig mit dem Hämoglobin.

Tabelle 8. Kaninchenserum wurde auf $\frac{1}{50}$ verdünnt und die Hälfte des verdünnten Serums dann eine Stunde erhitzt. Bei dieser Verdünnung koaguliert das Serum durch Kochen nicht, und die Flüssigkeit bleibt vollkommen klar. In dem angeführten Beispiel wurde das Serum nicht vorher dialysiert, aber Versuche mit eine Woche lang dialysiertem Serum haben praktisch ähnliche Resultate geliefert. Bei einer Verdünnung von $\frac{1}{50}$ beträgt der Salzgehalt nicht mehr als 0.045% oder ungefähr $\frac{1}{130}$ Normalität, eine Konzentration, welche keine beachtenswerte, erweiternde Wirkung auf die Ausflockungszone ausübt. Das Serum wird in Übereinstimmung mit der Regel ausgeflockt. Die Unterschiede zwischen dem nicht erhitzten und dem erhitzten Serum werden später erörtert werden.

Tabelle 9.

Mikroskopische Suspensionen.

Typhusbazillen: normal und Agglutinin.

Nr.	$Fe(OH)_3$ und basische Farbstoffe. Endgültige Ver- dünnungen %	Normaltyphusbazillen						Typhusagglutinin					
		$Fe(OH)_3$	Nacht- blau	Janus- grün	Nilblau	Neutral- rot	Methylen- blau	$Fe(OH)_3$	Nacht- blau	Janus- grün	Nilblau	Neutral- rot	Methylen- blau
1	$\frac{1}{30}$	—	—	—	—	—	—	—	—	—	—	—	Spur?
2	$\frac{1}{40}$	—	—	—	—	—	—	—	—	—	—	—	+
3	$\frac{1}{60}$	—	—	—	—	—	—	—	—	—	—	—	+
4	$\frac{1}{80}$	—	—	—	—	—	—	—	—	—	—	—	+
5	$\frac{1}{100}$	—	—	—	—	—	—	—	—	—	—	—	+
6	$\frac{1}{120}$	—	—	—	—	Spur	—	—	—	—	—	—	+
7	$\frac{1}{140}$	+++	—	—	+	+	—	—	—	—	—	—	+
8	$\frac{1}{160}$	+++	+	+++	+	+	—	—	—	—	—	+++	+
9	$\frac{1}{180}$	+++	+	+++	+	+	—	—	—	—	—	+++	+
10	$\frac{1}{200}$	+++	+++	+++	+	+	—	—	—	—	—	+++	+
11	$\frac{1}{240}$	+++	+++	+++	+	++	—	—	+	++	Spur	+++	—
12	$\frac{1}{280}$	+++	—	+	++	+	—	—	++	+++	Spur	++	—
13	$\frac{1}{320}$	—	—	Spur	—	—	—	—	+++	+++	+	++	—
14	$\frac{1}{360}$	—	—	—	—	—	—	—	++	+++	++	++	—
15	$\frac{1}{400}$	—	—	—	—	—	—	—	+	+++	++	Spur	—
16	$\frac{1}{480}$	—	—	—	—	—	—	++	Spur	+++	—	++	—
17	$\frac{1}{560}$	—	—	—	—	—	—	+	—	+++	—	Spur	—
18	$\frac{1}{640}$	—	—	—	—	—	—	—	—	++	—	—	—
19	$\frac{1}{720}$	—	—	—	—	—	—	—	—	—	—	—	—
20	$\frac{1}{800}$	—	—	—	—	—	—	—	—	—	—	—	—
21	$\frac{1}{960}$	—	—	—	—	—	—	—	—	—	—	—	—
22	$\frac{1}{1120}$	—	—	—	—	—	—	+++	—	—	—	—	—
23	$\frac{1}{1280}$	—	—	—	—	—	—	+++	—	—	—	—	—
24	$\frac{1}{1600}$	—	—	—	—	—	—	+++	—	—	—	—	—
25	$\frac{1}{1920}$	—	—	—	—	—	—	+++	—	—	—	—	—
26	$\frac{1}{2300}$	—	—	—	—	—	—	—	—	—	—	—	—

Tabelle 9. Bakterien und Agglutininbakterien. Typhusbazillen, in 0.5% iger Formaldehydlösung suspendiert und dialysiert. Ein Teil der

dialysierten Bakterien wurde mit verdünntem, dialysiertem Immunsrum behandelt und durch Zentrifugieren gründlich gereinigt. Die so behandelten Bakterien nennen wir „Agglutininbakterien“. Für den in der Tabelle angeführten Versuch wurden die beiden Suspensionen in möglichst gleicher Stärke hergestellt. Die Zonen für die hochkolloidalen Farbstoffe sind in beiden Fällen etwas weit, aber die Ausflockung ist bei gewissen Konzentrationen vollständig, während von den wenig kolloidalen Farbstoffen die Normalbakterien sehr wenig in Mitleidenschaft gezogen werden; die Agglutininbakterien indessen deutlich stärker.

Tabelle 10.

Russ, Gelatine und Stärke.

Nr.	$Fe(OH)_3$ und basische Farbstoffe. Endgültige Verdünnungen ‰	Russ				Stärke				Gelatine 1/4 ‰	
		$Fe(OH)_3$	Janus- grün	Nilblau	Methylen- blau	$Fe(OH)_3$	Janus- grün	Nilblau	Methylen- blau	Nacht- blau	Neutral- rot
1	1/26	—	—	++	—	—	—	++	Spur	—	—
2	1/40	—	—	++	—	—	—	++	Spur	—	—
3	1/60	—	—	++	—	+++	—	++	Spur	—	—
4	1/80	+++	—	++	—	+++	—	++	Spur	—	—
5	1/100	+++	—	++	—	+++	—	++	Spur	—	—
6	1/120	—	—	++	—	—	—	++	Spur	—	—
7	1/140	—	—	+	—	—	—	++	Spur	—	—
8	1/160	—	—	+	—	—	—	++	—	—	—
9	1/180	—	—	+	—	—	—	++	—	—	—
10	1/200	—	+++	—	—	—	—	++	—	—	—
11	1/240	—	—	—	—	—	—	++	—	—	—
12	1/280	—	—	—	—	—	—	+	—	—	—
13	1/320	—	—	—	—	—	++	—	—	+++	—
14	1/360	—	—	—	—	—	+++	—	—	+++	—
15	1/400	—	—	—	—	—	+++	—	—	—	—
16	1/480	—	—	—	—	—	+++	—	—	—	—
17	1/560 — 1/∞	—	—	—	—	—	—	—	—	—	—

Tabelle 10. Russ, Stärke und Gelatine. Diese drei Stoffe erfordern keine besondere Bemerkung und sind in eine Tabelle zusammengefasst worden, um zu zeigen, dass sie sich der Regel anpassen.

Allgemeine Erörterung der Tabellen 2 bis 10.

Die Tabellen zeigen sehr deutlich, dass die Regel der kolloidalen Ausflockung im allgemeinen gültig ist, und unter der Annahme, dass die Regel verlässlich ist, können wir sie zur Beurteilung des relativen Grades der kolloidalen Natur der untersuchten Substanzen verwenden, indem wir von der uns bereits bekannten relativen kolloidalen Natur der Farbstoffe zurückschließen.

Einige der untersuchten Kolloide zeigen mit den hochkolloidalen Farbstoffen sehr enge Ausflockungszonen. Das zeigt sich besonders deutlich bei Mastix, kolloidalem Platin und Arsensulfid, so dass wir diese Stoffe als in einem hochkolloidalen Zustand befindlich betrachten können. Andererseits zeigen Normalserum, Hämoglobin und besonders Tannin verhältnismässig weite Ausflockungszonen mit den hochkolloidalen und geringe oder gar keine Ausflockung mit den wenig kolloidalen Farbstoffen, und es kann daher geschlossen werden, dass diese Stoffe selbst relativ wenig kolloidal sind.

Indem wir diese Betrachtungsweise weiter führen, finden wir, dass obgleich die Ausflockungszonen von Lecithin, Gelatine und Stärke mit hochkolloidalen Farbstoffen eng sind, diese Kolloide doch durch wenig kolloide Farbstoffe unvollkommen oder gar nicht ausgeflockt werden. Diese Stoffe zusammen mit erhitztem Serum und Bakterien scheinen eine Mittelstellung einzunehmen, welche derjenigen von Nilblau und Biebricher Scharlach entspricht, und sie könnten daher als mässig kolloidal klassifiziert werden.

Höber¹⁾ gibt die folgenden durch Siede- oder Gefrierpunktmessungen annähernd bestimmten Molekulargewichte:

Stärke	25000
Eiweiss (aus Eiern)	14000
Tannin	3500.

Wir haben Stärke als mässig kolloidal und Serumeiweiss als wenig kolloidal, aber doch deutlich stärker kolloidal als Tannin, betrachtet.

Die Beobachtungen über Hämoglobin, Serum und Bakterien seien in diesem Zusammenhang weiter erörtert. Obgleich das Eiweiss des Hämoglobins und des Serums, nach unserm Massstab beurteilt, relativ wenig kolloidal zu sein scheint, so wissen wir doch, dass es nicht durch Pergamentpapier dialysiert, und dieses Verhalten bei der Dialyse kann auf die Grösse der einzelnen Moleküle zurückgeführt werden. Vom Hämoglobin nimmt man, nach Schätzungen von chemischen Gesichtspunkten aus, an, dass es ein Molekulargewicht von ungefähr 14000 besitzt. Dies entspricht dem Molekulargewicht des Hühnereiweisses, wie es sich nach der Gefrierpunktserniedrigung ergibt, eine Übereinstimmung, die darauf hindeutet, dass Eiweissstoffe ihre kolloidalen Eigenschaften eher der Grösse, als der Aggregation ihrer Moleküle verdanken. Man wird daher von ihnen erwarten, dass sie sich hinsichtlich der Ausflockung mehr wie die wenig kolloidalen, als wie die hoch-

¹⁾ Physikalische Chemie der Zelle.

kolloidalen Farbstoffe verhalten werden, für welch letztere eher die Aggregation als die Grösse der Moleküle der Hauptfaktor bei der Ausflockung zu sein scheint.

Die Betrachtung der Tabelle 8 (Serum nicht erhitzt und erhitzt) zeigt sehr deutlich, dass Erhitzen die Ausflockungszonen für die hochkolloidalen Farbstoffe einengt und das Ausflockungsvermögen der schwachkolloidalen Farbstoffe erhöht, bis zu einem gewissen Grade eine Bestätigung unserer Behauptung, denn es ist allgemein anerkannt, dass das Kochen des Serums die Grösse der Eiweissaggregate erhöht und daher — in Dreapers¹⁾ Ausdrucksweise — die Löslichkeit degradiert. Bei hoher Konzentration des Serums zeigt sich diese Degradation der Löslichkeit durch Koagulation an, bei hoher Verdünnung aber nur durch eine Erhöhung der Grösse der Aggregate, ohne sichtbare Veränderung.

Wenden wir uns der Tabelle 9 über Bakterien und Agglutininbakterien zu, so finden wir, dass, obgleich mit den hochkolloidalen Farbstoffen die Ausflockungszonen in der Tabelle viel tiefer liegen, sie doch ungefähr ebenso breit sind, wie im Falle der Normalbakterien. Diese Tatsache scheint darauf hinzudeuten, dass die Grösse der Aggregate nicht zugenommen hat, und das ist zu erwarten, da in destilliertem Wasser suspendierte Agglutininbakterien erst nach Zusatz eines Elektrolyten Neigung zur Ausflockung zeigen. Field und Teague²⁾ haben gezeigt, dass Typhusagglutinin im elektrischen Strom zur Kathode wandert, also eine positive Ladung trägt, und wir haben häufig beobachtet, dass Agglutininbakterien zur Anode wandern, obgleich weniger bereitwillig wie Normalbakterien. Die niedrigere Stellung der Ausflockungszonen der Agglutininbakterien in Tabelle 9 mag daher rühren, dass die elektrischen Ladungen durch das Agglutinin teilweise neutralisiert wurden, so dass weniger vom positiven Kolloid erforderlich ist, um Aggregation mit darauffolgender Ausfällung hervorzurufen, als bei Normalbakterien nötig gewesen wäre.

Vom Standpunkte der Regel der kolloidalen Ausflockung nehmen wir als Unterschied zwischen erhitztem Serum und Agglutininbakterien an, dass beim erhitzten Serum die mit dem Farbstoffe reagierenden ursprünglichen Aggregate grösser sind, als beim normalen Serum, so dass ein Überschuss des erhitzten Serums besser imstande ist, den Niederschlag aufzulösen, als ein Überschuss des normalen Serums. Im Falle der Agglutininbakterien sind die ursprünglichen, mit dem Farbstoff reagierenden Aggregate nicht grösser als bei den Normalbakterien,

¹⁾ Journ. Soc. Chem. Industry 24, 223 (1905).

²⁾ Journ. Exp. med. 9, 86 (1907).

so dass ein Überschuss den Niederschlag nicht leichter auflöst, als ein Überschuss von Normalbakterien.

Nachdem wir nun, wie wir glauben, die Gültigkeit der Regel der kolloidalen Ausflockung festgestellt haben, können wir zu einem andern Punkt im Verhalten der Farbstoffe übergehen, welcher sich allmählich unserer Aufmerksamkeit aufgedrängt hat, dass nämlich im Optimalpunkte der Ausflockung die beiden Farbstoffe in äquimolekularen Mengen vorhanden sind, und das Salz quantitativ in Freiheit gesetzt wird. Bayliss¹⁾ und andere haben beobachtet, dass bei der Herstellung der neutralen Methylenblau-Eosinfarbe *Na* und *Cl* quantitativ frei werden. Es ist auch nach Georgevicz festgestellt, dass beim Färben von Gespinsten mit basischen oder sauren Farbstoffen die Säure, resp. das Alkali, in Freiheit gesetzt wird, und um das Freiwerden der Farbbase oder -säure zu unterstützen, werden die Gespinste für saure Färbung in einem sauren Bad und für basische Färbung in einem alkalischen Bad behandelt.

Matthews²⁾ scheint der erste gewesen zu sein, der die Gründe für eine solche Behandlung bei histologischen Färbungen untersuchte und erläuterte.

Wenngleich wir nicht versucht haben, genau nachzuweisen, dass bei der gegenseitigen Fällung zweier Farbstoffe in äquimolekularem Verhältnis das Salz quantitativ in Freiheit gesetzt wird, so haben wir doch genügend viel rohe Versuche angestellt, die dies wahrscheinlich machen. Unser Augenmerk war hauptsächlich darauf gerichtet, nachzuweisen, dass wenn vollkommene Ausfällung zweier Farbstoffe eintritt, sie immer in äquimolekularem oder annähernd äquimolekularem Verhältnis vorliegen. Als Beispiel sei folgendes erwähnt: Kongorot, mit dem Molekulargewicht 698 ist in $\frac{1}{200}$ %iger Lösung $\frac{1}{13960}$ -mol., und da Kongorot zwei Natriumsulfogruppen besitzt, so würde die äquimolekulare Lösung von Nachtblau $\frac{1}{6980}$ -mol. in bezug auf letzteres sein. Wählen wir Nachtblau, so würde der optimale Ausflockungspunkt in $\frac{575}{6980} \times \frac{1}{10}$ oder $\frac{1}{121.5}$ %iger Lösung liegen, wenn das Kongorot $\frac{1}{200}$ %ig ist. Indem wir die Konzentration von $\frac{1}{122}$ % als „Mittelpunkt“ wählten, stellten wir mit engen Intervallen eine Reihe von Lösungen verschiedener Konzentration her, die mit dem Kongorot gemischt wurden. Die Resultate sind in Tabelle 11, Kolonne 1 enthalten. Die Ausflockung wurde zuerst

¹⁾ Biochemical Journ. 1, 175 (1906).

²⁾ Am. Journ. of Phys. 1, 445 (1898).

in der $\frac{1}{124}\%$ igen Lösung sichtbar und war bei dieser Konzentration nach 24 Stunden vollständig, so dass die überstehende Flüssigkeit farblos war. In $\frac{1}{120}\%$ - und $\frac{1}{128}\%$ iger Lösung dagegen war die Flüssigkeit, obgleich scheinbar vollkommene Ausflockung stattgefunden hatte, gefärbt, ein Zeichen dafür, dass überschüssiger Farbstoff in der Lösung vorhanden war. Die Regel des äquimolekularen Verhältnisses wurde daher innerhalb der Fehlergrenzen befolgt.

Tabelle 11.

Enge Konzentrationsintervalle bei hochkolloidalen Farbstoffen.

Nr.	Nachtblau Endgültige Verdünnungen	Kongorot $\frac{1}{200}$	Trypanrot $\frac{1}{200}$	Janusgrün Endgültige Verdünnungen	Kongorot $\frac{1}{200}$	Trypanrot $\frac{1}{400}$
1	$\frac{1}{108}$	—	—	$\frac{1}{124}$	—	—
2	$\frac{1}{112}$	—	—	$\frac{1}{124}$	—	—
3	$\frac{1}{116}$	+	—	$\frac{1}{128}$	—	—
4	$\frac{1}{120}$	+++	+++ c	$\frac{1}{128}$	—	+++ c
5	$\frac{1}{124}$	+++ c	+++ c	$\frac{1}{128}$	—	+++ c
6	$\frac{1}{128}$	+++	+++ c	$\frac{1}{144}$	—	+++ c
7	$\frac{1}{132}$	+	—	$\frac{1}{144}$	+++ c	—
8	$\frac{1}{136}$	+	—	$\frac{1}{152}$	+++ c	—
9	$\frac{1}{140}$	+	—	$\frac{1}{156}$	—	—
10	$\frac{1}{144}$	—	—	$\frac{1}{160}$	—	—

c bedeutet, dass die überstehende Flüssigkeit farblos war.

Ebenso haben wir eine in dieser Abhandlung nicht mitgeteilte Tabelle über Nilblau und Eosin zusammengestellt, nach welcher sich für Eosin (692) in $\frac{1}{200}\%$ iger Lösung Ausflockung mit Nilblau (350) in $\frac{1}{160}\%$ -, $\frac{1}{200}\%$ -, $\frac{1}{240}\%$ - und $\frac{1}{320}\%$ iger Lösung ergibt, am vollständigsten in $\frac{1}{200}\%$ - und $\frac{1}{240}\%$ iger Lösung. Da Eosin wenig und Nilblau mässig kolloidal ist, ist die Zone breit, aber theoretisch sollte die Optimalkonzentration bei $\frac{1}{226}\%$ liegen.

Es könnten noch weitere Beispiele angeführt werden, und die Tatsache, dass im Punkte der vollkommensten Ausflockung die Farbstoffe in angenähert äquimolekularen Mengen vorliegen, ist so häufig beobachtet worden, dass sie wahrscheinlich regelmässig eintritt, obgleich wir einige scheinbare Ausnahmen angetroffen haben, in Fällen, in denen einer der Farbstoffe, wie Alkaliblau oder Azoblau, nur wenig löslich ist. In solchen Fällen zeigte sich der wenig lösliche Farbstoff immer etwas verdünnter, als unsere tabellierten Prozentzahlen (in einer frühern Mitteilung) andeuten. Wahrscheinlich sind derartige Farbstoffe nicht völlig in Lösung gegangen.

In einigen andern Fällen haben wir Abweichungen zwischen den in den Büchern angegebenen und den nach unsern Versuchen zu er-

wartenden Molekulargewichten gefunden. Janusgrün z. B. ist nach Schulze und Julius¹⁾ ein mit einem diazotierten Safraninradikal verbundenen Dimethylanilin; das würde einem Molekulargewicht von ungefähr 470 entsprechen. Janusgrün wird aber durch Kongorot und andere saure Farbstoffe bei einer Konzentration ausgeflockt, die darauf hinweist, dass es ein Molekulargewicht von ca. 695 hat. So z. B. flockt $\frac{1}{300}$ %iges = $\frac{1}{20940}$ -molares Kongorot (Tabelle 11, Kolonne 3) $\frac{1}{148}$ - und $\frac{1}{152}$ %iges Janusgrün aus, aber mit $\frac{1}{144}$ - und $\frac{1}{156}$ %igem Janusgrün erfolgt keine Ausflockung, so dass die $\frac{1}{150}$ %ige Lösung als Optimalpunkt angesehen werden kann. Da ein (Kongorot—2 Na) zwei (Janusgrün—Cl) äquivalent ist, sollte das Molekulargewicht des letztern annähernd 698 sein, gleich dem Molekulargewicht von Kongorot.

Des weitern wurde der Optimalpunkt für Eosin (692) ungefähr bei $\frac{1}{400}$ - bis $\frac{1}{200}$ % Janusgrün gefunden, so dass letzteres ein Molekulargewicht von ungefähr 692, oder ein ebenso grosses wie Eosin, haben sollte. Das Molekulargewicht von Janusgrün scheint daher in der Nähe von 695 zu liegen.

Biebricher Scharlach sei hier ebenfalls erwähnt. Nach Schulze und Julius ist er ein Disazofarbstoff mit zwei Sulfogruppen und sollte ein Molekulargewicht von 546 haben. Nach Nietzki dagegen ist der Biebricher Scharlach des Handels häufig ein Gemisch der Disulfo- und Monosulfosalze, und bei unsern Versuchen hat er sich immer so verhalten, als bestände er ausschliesslich aus dem Monosulfosalz, welchem ein Molekulargewicht von 444 zukommt. Seine optimalen Ausflockungspunkte mit verschiedenen basischen Farbstoffen — die Details brauchen hier nicht angeführt zu werden — deuten darauf hin, dass das Molekulargewicht des Biebricher Scharlachs annähernd 444 und nicht 546 ist.

In Tabelle 11 sind einige Ausflockungsversuche mit Trypanrot angeführt. Trypanrot von dem Molekulargewicht 1000 hat fünf Natriumsulfogruppen, aber die optimalen Ausflockungspunkte mit Nachtblau (575) und Janusgrün (zu 695 gerechnet) deuten an, dass eine dieser Gruppen unwirksam ist. Wenn dies zutrifft, sollte der Optimalpunkt mit Nachtblau bei $\frac{1}{180}$ % und mit Janusgrün bei $\frac{1}{144}$ % liegen, welche Konzentrationen den in der Tabelle angegebenen Optimalpunkten von $\frac{1}{124}$ und $\frac{1}{140}$ % sehr nahe kommen. Wären alle fünf Sulfogruppen wirksam, so sollten die Optimalpunkte bei $\frac{1}{104}$ und $\frac{1}{115}$ % liegen, also weit von den tatsächlich beobachteten.

Obleich es scheint, dass zwei Farbstoffe in ihrem optimalen Aus-

¹⁾ Organic coloring matters, übersetzt von Green, 1904.

flockungspunkte ungefähr in äquimolekularen Mengen zugegen sind, so gilt diese Regel nicht für die Ausflockung von andern Kolloiden durch Farbstoffe. Das wird durch Tabelle 12 gezeigt. Der optimale Ausflockungspunkt für $\frac{1}{14000}$ -mol. Kongorot durch die basischen Farbstoffe findet sich ungefähr in $\frac{1}{7000}$ -mol. Lösung der letztern, aber für Mastix, Lecithin, kolloidales Platin und erhitztes Serum variiert die zur optimalen Ausfällung erforderliche Konzentration der Farbstoffe beträchtlich. Es scheint nach den in der Tabelle angegebenen Resultaten, in diesen Fällen im allgemeinen, zur Bewirkung vollständiger Ausfällung

Tabelle 12.

Annähernde Konzentration bei den Optimalpunkten der Ausflockung.

	Kongorot $\frac{1}{200} = M/14000$	Mastix	Kolloidales Platin	Lecithin	Erhitztes Serum
1. Nachtblau	$M/7000$	$M/60000$	$M/70000$	$M/32000$	$M/5750$
2. Janusgrün	$M/7000$	$M/110000$	$M/120000$	$M/16000$	$M/6000$
3. Nilblau	$M/7000$	$M/60000$	$M/70000$	$M/12000$	$M/3500$
4. Neutralrot	$M/7000$	$M/20000$	$M/50000?$	$M/2000?$	$M/2000?$
5. Methylenblau	$M/7000?$	$M/6000$	$M/40000?$	Keine Ausflockung	$M/2500?$

eine um so grössere Menge des Farbstoffs erforderlich zu sein, je weniger kolloidal er ist. Allerdings lässt sich dies wegen der Schwierigkeit der Ermittlung der ungefähren Optimalpunkte, besonders bei den weniger kolloidalen Farbstoffen, nicht mit Sicherheit behaupten. Die obige Ansicht wird indessen durch Tabelle 12 insofern weiter bekräftigt, als zur Ausflockung von Lecithin und erhitztem Serum eine grössere Menge des Farbstoffs erforderlich ist, als zu derjenigen von Mastix oder kolloidalem Platin. Die beiden erstern Stoffe sind schon unter die mässig kolloidalen, die beiden letztern unter die hochkolloidalen Substanzen klassifiziert worden.

Einfluss des in Freiheit gesetzten Salzes auf die Ausflockung.

Es ist schon früher sichergestellt und durch die Tabellen in einer unserer Abhandlungen erhärtet worden, dass der Zusatz von Natriumchlorid die Zone der kolloidalen Ausflockung beträchtlich erweitert, und es ist wahrscheinlich, dass bei der gegenseitigen Ausflockung zweier Farbsalze das in Freiheit gesetzte Natriumchlorid einen erheblichen Einfluss nach dieser Richtung hin ausübt. Unsere Aufmerksamkeit wurde auf diese Erscheinung zuerst durch das Verhalten des Farbstoffgemisches Nachtblau plus Kongorot gelenkt. Als wir den Niederschlag in Methylalkohol auflösten und etwas von der Lösung in destilliertes

Wasser einbrachten, fanden wir zu unserer Überraschung, dass keine Ausflockung eintrat, selbst nachdem der ganze Alkohol verdampft war. Unter diesen Bedingungen können nur winzige Spuren von Elektrolyten zugegen sein, so dass der geringste Überschuss eines der Farbstoffe, welcher wahrscheinlich im Niederschlag als Salz vorhanden ist, ausreichen würde, um die Verbindung in Lösung zu halten. Die Farbstoffe wurden daher so nahe wie möglich in äquimolekularen Mengen gemischt. Kongorot $\frac{1}{200}$ %ig und Nachtblau $\frac{1}{121.5}$ %ig wurden zusammen gefällt. Ein Teil des Niederschlages wurde nicht ausgewaschen, der andere Teil im Dampfterilisierapparat zwei oder drei Stunden lang mit kochendem Wasser gewaschen, um einen etwaigen geringen Überschuss des einen Farbstoffs zu entfernen. Die Niederschläge wurden dann in Methylalkohol bis zur Sättigung gelöst. Zu 10 ccm Wasser wurden einige Tropfen der Methylalkohollösung hinzugefügt, aber auch hier stellte sich heraus, dass in keinem der Röhrchen Ausflockung eintrat. Nach Zusatz weniger Tropfen Salzlösung zu jedem Röhrchen trat jedoch in der Lösung des ausgewaschenen Niederschlages rasche Ausflockung ein, und die Fällung war nach einigen Stunden vollständig. Der nicht gewaschene Teil hingegen zeigte selbst nach 24 Stunden nur spurenhafte Ausflockung. Wir nehmen an, dass der ausgewaschene Niederschlag die beiden Farbstoffe sehr nahe aber doch nicht ganz in äquimolekularen Mengen enthielt, so dass eine Spur Salz notwendig war, um die Wirkung des im Überschuss vorhandenen Farbstoffs zu überwinden und die Ausflockung einzuleiten. Der Versuch wurde mehrmals mit ähnlichen Ergebnissen wiederholt.

Bei Verwendung des weniger kolloidalen Nilblaus, das mit Kongorot eine weitere Ausflockungszone gibt, als Nachtblau und nach Behandlung des Niederschlages in der gleichen Weise, flockte der ausgewaschene Teil sofort ohne Zusatz von Salz aus, während mit dem nicht gewaschenen Teil keine Ausflockung eintrat.

Weitere Versuche in derselben Richtung bestätigten ebenfalls die Ansicht, dass bei der gegenseitigen Ausflockung von Farbstoffen genügend Salz in Freiheit gesetzt wird, um die Ausflockungszone in merklichem Betrage zu erweitern. Die Reaktion zwischen Kongorot und Nilblau ist: (Kongorot : $2Na$) + 2 (Nachtblau . Cl) = Kongorot . 2Nachtblau + $2NaCl$.

Histologisches.

Unsere früher beschriebenen Versuche mit Farbstoffgemischen unter dem Einflusse des elektrischen Stroms und unter andern Bedingungen zeigten uns ziemlich sicher, dass zwei hochkolloidale Farbstoffe viel

fester miteinander verbunden sind, als zwei wenig kolloidale, und Färbeversuche scheinen diese Ansicht zu bestätigen. Zu den Färbeversuchen benutzten wir hauptsächlich das Omentum von Kaninchen. Es wurde auf einem mikroskopischen Objektglas ausgebreitet, mit Methylalkohol befestigt und das Salz soweit wie möglich durch wiederholtes Behandeln mit destilliertem Wasser ausgewaschen. Die so behandelten Präparate wurden mit einer Lösung des Farbstoffkomplexes in Methylalkohol, dem einige Tropfen destilliertes Wasser zugesetzt worden waren, bedeckt, eine der gewöhnlichen Methylenblau-Eosinfärbung ähnliche Technik. Bei den kolloidalen Farbstoffen war es nötig, einen von ihnen in geringem Überschuss anzuwenden, um Ausfällung auf dem Präparat zu verhindern.

1. Nachtblau—Kongorot (beide hochkolloidal) keine Färbung selbst nach mehrern Stunden, manchmal blosse Spuren.

2. Nachtblau (hoch-) —Eosin (wenig kolloidal). Das Präparat färbte sich ziemlich langsam blau. Nach dem Ausziehen des Nachtblaus mit Alkohol blieb eine blasser Eosinfärbung zurück. Das hochkolloidale Nachtblau wird durch das Eosin nicht fest zurückgehalten, und gleichzeitig wird etwas Eosin frei.

Das Präparat färbt sich also, aber ziemlich unvollkommen.

3. Methylenblau—Eosin (beide wenig kolloidal). Wie wohl bekannt, färben sich Präparate mit diesem Gemisch sehr bereitwillig und selektiv, und dies traf auch bei uns zu. Die beiden wenig kolloidalen Farbstoffe halten einander nur in loser Verbindung.

Diese Versuche sind in vielen andern Fällen bestätigt worden.

Es scheint möglich, dass ein hochkolloidaler Stoff sich von einem wenig kolloidalen trennen kann, um sich mit einem kolloidalen zu verbinden. Bei den histologischen Färbeversuchen sind die hochkolloidalen Eiweissstoffe vielleicht imstande, das Nachtblau dem wenig kolloidalen Eosin zu entziehen, aber sie können nicht das Nachtblau von dem hochkolloidalen Kongorot trennen.

Wie früher erwähnt, betrachten wir allerdings infolge der Resultate unserer Versuche die Eiweissstoffe in Lösung als relativ wenig kolloidal, aber der Zustand des Eiweisses in Lösung ist kein Kriterium für seinen Zustand im Gewebe.

Sind die gefällten Farbstoffe chemisch oder physikalisch gebunden?

Diese Frage mag kurz erörtert werden, soweit die Ergebnisse unserer Versuche darauf Bezug zu haben scheinen. Die Tatsache, dass zwei Farbstoffe sich in äquimolekularen Mengen verbinden, während

Na und *Cl* quantitativ in Freiheit gesetzt werden, macht es auf den ersten Blick wahrscheinlich, dass die Verbindung der beiden Farbstoffe chemischer Natur ist, da aber die aus zwei hochkolloidalen Farbstoffen gebildete Verbindung viel beständiger ist, als die aus zwei wenig kolloidalen Farbstoffen gebildete, so haben wir Grund zur Annahme, dass es sich um eine auf gegenseitiger Absorption beruhende physikalische Bindung handelt, die durch die chemische Verbindung von *Na* und *Cl* begünstigt wird.

NaOH kann das *Cl* aus dem Salz (Chlorid) eines basischen Farbstoffs austreiben, wobei die Base als unlöslicher Niederschlag ausfällt, und umgekehrt werden Säuren, die Säure aus dem Salz einer Farbsäure austreiben. Wenn solche Farbsalze zusammen in Wasser aufgelöst werden, so tritt wahrscheinlich etwas Dissociation, ein und es wird fortwährend *NaCl* auf Kosten der Farbsalze gebildet. Die in Freiheit gesetzten Basen und Säuren fallen dann aufeinander aus und lassen das *NaCl* in der Lösung. Da das Farbstoffgemisch aus der Lösung entfernt ist, kann es sich an den Reaktionen nicht weiter beteiligen, und die Farbstoffe fallen in äquimolekularen Mengen aus, nicht weil sie notwendig selbst in solchem Verhältnis chemisch verbunden sind, sondern weil der Rückstand nach der Bildung von *NaCl* in einem solchen Verhältnis vorliegen muss.

Die Verbindung zweier hochkolloidaler Farbstoffe scheint ihrer Natur nach den Lacken sehr nahe zu kommen. Sie wird schwierig aufgespalten. Sogar durch Kochen mit Säure oder Alkali lässt sich nur eine geringe Spur des einen Farbstoffs in Gestalt eines Salzes herauslösen. Diesen Widerstand der Lacke gegen Säuren und Alkalien betrachtet Georgevics als starken Beweisgrund für die Ansicht, dass ein Lack eine physikalische Verbindung ist, und dieselbe Betrachtungsweise wäre auf die Farbstoffkomplexe anwendbar.

Schlussfolgerungen.

1. Die Ausflockung saurer Farbstoffe durch basische ist am vollständigsten, wenn die Farbstoffe in äquimolekularen Mengen vorliegen.
2. Bei der Ausflockung wird Natriumchlorid in Freiheit gesetzt, doch ist dies nicht die Ursache der Ausflockung; allerdings ist das Salz bestrebt, die Ausflockungszone zu erweitern.
3. Färbungsversuche mit Farbstoffgemischen deuten an, dass hochkolloidale Farbstoffe fester miteinander verbunden sind als wenig kolloidale.

4. Mit einer neuen Reihe von Farbstoffen wurden die in Teil IV erhaltenen Resultate bestätigt: hochkolloidale Farbstoffe haben enge Zonen vollkommener Ausflockung, wenig kolloidale Farbstoffe zeigen unvollkommene Ausflockung innerhalb einer ausgedehnten Zone.

5. Wir haben dieses Verhalten als die Regel der kolloidalen Ausflockung bezeichnet: Der Grad der Ausflockung und die Breite der Ausflockungszone werden durch die mehr oder weniger kolloidale Natur der verwendeten Stoffe bedingt.

6. Die Regel der kolloidalen Ausflockung ist für verschiedene andere anorganische und organische Kolloide sowohl als wie für die Farbstoffe als gültig erwiesen worden.

Einfluß der Temperatur auf die Ausflockung von Kolloiden.

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Die Frage nach der Hydratation der Ionen in wässrigen Lösungen hat neuerdings viel Beachtung gefunden. Namentlich haben Biltz¹⁾ und Jones²⁾ eine überaus große Zahl von Versuchen ausgeführt, um die Abhängigkeit des Hydratationsgrades von der Konzentration der Lösung zu zeigen.

Bousfield³⁾ ermittelte durch Messungen, daß sowohl Temperatur als Konzentration einen erheblichen Einfluß darauf ausüben. Setzt man z. B. das Volumen des Wasserstoffions bei 20° gleich 1, so ist es bei 0° annähernd 0,8, bei 40° annähernd 1,2 und nimmt über 40° wieder ab.

Es kam uns der Gedanke, daß die wechselnde Hydratation des H-Ions vielleicht mit dem Phänomen der Vorzone zusammenhängt, das so oft bei der Ausflockung von Kolloiden durch Säuren beobachtet wird, und wir stellten darum eine Anzahl von Ausflockungsversuchen mit Säuren und Salzen bei wechselnder Temperatur an. Im ganzen fanden wir, daß mit Steigerung der Temperatur die Breite der Ausflockungszone zunahm, und Bakterien zeigten auf Säurezusatz bei 40° gelegentlich eine Neigung zum Auftreten von unregelmäßigen Reihen, die bei 0 und 20° nicht vorhanden war. Immerhin haben unsere Versuche keinen Zusammenhang zwischen dem angenommenen Hydratationsgrad des H-Ions und der Breite der Vorzonen bei verschiedenen durch

¹⁾ Zeitschr. f. physik. Chem. 40, 185 (1902).

²⁾ Amer. Chem. Journ. 33, 34 (1905) und andere Journale.

³⁾ Zeitschr. f. physik. Chem. 53, 257 (1906).

Säuren ausflockbaren Kolloiden ergeben; sie sollen daher hier nicht näher mitgeteilt werden.

Wenn wir nun zu Versuchen über die Ausflockung von Kolloiden durch Kolloide übergehen, haben wir über bemerkenswerte Ergebnisse zu berichten, die wir bei weitem nicht für aufgeklärt ansehen, wenngleich wir auf Grund von vorläufigen Arbeits-hypothesen im nachstehenden einige Deutungsversuche machen.

Wir benutzten in diesen Versuchen Probierröhrchen von 9 cm Länge, die zu etwa 20 Stück in mit Gewichten beschwerten Eprouvettengestellen untergebracht waren. In die Röhrchen wurde 1 ccm der einen kolloidalen Lösung von wechselnder Konzentration eingefüllt und das Gestell in ein Wasserbad von der gewünschten Temperatur versenkt. Die andere Kolloidlösung wurde im ganzen durch Erwärmen oder Abkühlen auf die gleiche Temperatur gebracht und je 1 ccm davon mittels einer Maßpipette in die Röhrchen zu der ersten Kolloidlösung zugesetzt. Durch etwas heftiges Ausblasen der Pipette wurde eine genügende Mischung der beiden Flüssigkeiten erzielt, so daß kein Schütteln nötig war. Für die Temperaturen 0, 20 und 40° wurden systematische Versuchsreihen mit 1-, 3-, 5- und 24stündiger Dauer ausgeführt, Versuche bei 60 und 80° wurden nicht über 2 bis 3 Stunden ausgedehnt. Bei höherer Temperatur erfolgt die Flockenbildung so rasch, daß die Reaktion nach 2 Stunden praktisch zu Ende ist. Auch zeigte von den Versuchsreihen bei niedriger Temperatur nur jene bei 0° nach 4 Stunden noch eine merkliche Änderung. Bei den Reihen mit 0° und 40° wurden die Gestelle nach 5 Stunden aus dem Wasserbade genommen und in den Eiskasten oder den auf 38° erwärmten Brutraum gebracht. Bei 20° wurde vom Wasserbade abgesehen, doch war die Temperatur des mit Dampf geheizten Arbeitsraumes stets nahe 20°.

Ausflockung von Kolloiden durch Kolloide.

Zwischen 0 und 100° weisen viele Kolloidlösungen erhebliche Veränderungen auf. Einzelne erstarren bei niedriger Temperatur, z. B. Gelatine und Agar, andere bei höherer, z. B. Albumine. Die erstgenannte Veränderung ist reversibel, die letztere nicht. Das einmal durch Hitze koagulierte Eiweiß kann nicht mehr in kolloidale Lösung überführt werden, während Gelatine und Agar beliebig oft zum Erstarren und Schmelzen gebracht werden können¹⁾.

¹⁾ Lewites, Zeitschr. f. Chemie d. Kolloide 2, 16 (1908).

Wieder andere Kolloide werden innerhalb dieser Grenzen durch die Temperatur nicht beeinflusst. Eisenhydroxyd und kolloidales Platin können bis 0° abgekühlt und können gekocht werden, ohne daß Koagulation oder sichtbare Veränderung eintritt. Wenn sie aber — und ebenso manche andere künstlich erhältliche Kolloidlösungen — einmal durch andere Einwirkungen (Elektrolyte) zum Ausfällen gebracht worden sind, können sie nicht wieder durch Wasser allein in Lösung gebracht werden; dazu bedarf es dann eigener Methoden. Diese Kolloidlösungen sind somit irreversibel, obgleich ihre Haltbarkeit durch Temperaturen zwischen 0 und 100° nicht beeinflusst wird. Die Farbstoffe müssen danach, da sie getrocknet und dann jederzeit durch einfache Wasserzugabe wieder in kolloidale Lösung gebracht werden können, als reversible Kolloide angesehen werden¹⁾.

Wir können darnach die Kolloide in folgender Weise klassifizieren:

A. Zwischen 0 und 100° nicht koagulable Kolloide.

1. Reversibel: z. B. Farbstoffe.
2. Irreversibel: z. B. Eisenhydroxyd; kolloidales Platin.

B. Zwischen 0 und 100° koagulable Kolloide.

1. Reversibel: z. B. Gelatine, Agar, Stärke.
2. Irreversibel: z. B. Albumine.

Die von uns verwendeten Lösungen koagulabler Kolloide waren ausreichend verdünnt, um eine Ausfällung zwischen 0 und 100° zu vermeiden. Wir machten ferner auch von Bakterien- und Mastixsuspensionen Gebrauch.

Wir verzichten darauf, die Methoden, nach denen die verschiedenen von uns verwendeten Suspensionen und Lösungen dargestellt waren, näher zu beschreiben, nur sei bemerkt, daß zur Verdünnung durchweg gewöhnliches destilliertes Wasser verwendet wurde, und daß die Ausgangslösungen in jenen Fällen, wo sie notwendig Elektrolyten enthielten, z. B. bei einigen anorganischen Kolloiden und bei Bakteriensuspensionen, eine Woche lang der Dialyse gegen destilliertes Wasser unterworfen wurden.

Unser Hauptziel war stets die Bestimmung des Ausflockungspunktes, und dieser ist, wie uns die Erfahrung gelehrt hat, von der Gegenwart von Elektrolytspuren unabhängig. Die Ausflockungszone wird durch Anwesenheit merklicher Mengen von Elektrolyten verbreitert, das Optimum bleibt unverändert.

¹⁾ Biltz, Med.-naturwiss. Archiv 1, 267 (1908).

Farbstoffe.

Bei gegenseitiger Ausflockung zweier Farbsalze findet eine chemische Reaktion zwischen dem Kation des sauren und dem Anion des basischen Farbstoffs statt, so daß sich beim Optimum der Flockenbildung Säure und Base in äquimolekularem Verhältnis ausfällen. Es ist daher nicht zu erwarten, daß die Temperatur das Optimum beeinflusst. Das ergab auch der Versuch.

Tabelle I. Nachtblau und Biebricher Scharlach.
Endgültige Verdünnung des Nachtblau $\frac{1}{100}$ Proz.

Biebricher Scharlach Endverdünnung Proz.	0°	20°	40°	60°	80°
	24 Stdn.	24 Stdn.	24 Stdn.	3 Stdn.	1 Stde.
$\frac{1}{20}$ bis $\frac{1}{100}$	—	—	—	—	—
$\frac{1}{100}$	—	—	—	+	+
$\frac{1}{200}$	—	—	+++	++++	++++
$\frac{1}{250}$	+++	+++	+++	+++	+++
$\frac{1}{300}$	+	++	+	+++	+++
$\frac{1}{400}$ bis $\frac{1}{\infty}$	—	—	—	—	—

Der äquimolekulare Punkt zwischen den zwei Farbsalzen ist annähernd $\frac{1}{250}$ Proz. Biebricher Scharlach zu $\frac{1}{200}$ Proz. Nachtblau. Wie aus Tabelle I ersichtlich, liegt das Optimum in der Nachbarschaft dieses Punktes unabhängig von der Temperatur. Dieselbe Regelmäßigkeit ergab sich bei Versuchen mit Kongorot-Nachtblau, Kongorot-Nilblau und in einigen anderen Beispielen.

Nimmt man hingegen ein nicht salzartig verbundenes negatives Kolloid, das dem basischen Farbsalz gegenüber keinen scharfen Neutralisationspunkt besitzt, so erhält man ganz abweichende Resultate.

Tabelle II ist typisch für die Reaktion zwischen basischen Farbstoffen und dialysierten Bakterienaufschwemmungen: Als Ausflockungsoptimum, das durch Fettdruck ausgezeichnet ist, ist der Punkt bezeichnet, wo die Flockenbildung am frühesten beginnt und in der Beobachtungsreihe am meisten hervortritt. Es entspricht nicht notwendig der Mitte der Ausflockungszone.

Nachtblau und Janusgrün wurden für die Proben gewählt, weil beide mit den verschiedenen Kolloiden enge Ausflockungszonen darbieten und das Optimum der Flockenbildung leichter zu erkennen gestatten, als weniger kolloidale Farbstoffe, z. B. Neutralrot und Methylenblau, deren Ausflockungszonen gewöhnlich sehr breit sind.

Tabelle II. Einfluß der Temperatur auf die Ausflockung von Bakteriensuspensionen durch basische Farbstoffe.

Nachtblau Endverdünnung Proz.	Bac. pyocyaneus			
	0°	20°	40°	60° 2 Stdn.
$\frac{1}{20}$ bis $\frac{1}{50}$	—	—	—	—
$\frac{1}{100}$	—	—	++	+++
$\frac{1}{180}$	—	+	++	+++
$\frac{1}{140}$	—	+++	+++	+++
$\frac{1}{160}$	+	+++	—	—
$\frac{1}{200}$	+++	+	—	—
$\frac{1}{250}$	+++	—	—	—
$\frac{1}{300}$ bis $\frac{1}{\infty}$	—	—	—	—

Janusgrün Endverdünnung Proz.	Bac. coli comm.				
	0°	20°	40°	60° 2 Stdn.	80° 2 Stdn.
$\frac{1}{20}$ bis $\frac{1}{100}$	—	—	—	—	—
$\frac{1}{150}$	—	—	—	—	++
$\frac{1}{200}$	—	—	—	+	—
$\frac{1}{250}$	—	—	+++	—	—
$\frac{1}{300}$	—	—	+	—	—
$\frac{1}{400}$	—	+++	—	—	—
$\frac{1}{600}$	++	—	—	—	—
$\frac{1}{800}$ bis $\frac{1}{\infty}$	—	—	—	—	—

Man sieht, daß mit Steigerung der Temperatur mehr Farbstoff erforderlich ist, um eine bestimmte Menge von Bakterien auszuflocken. Es liegt nahe, zu vermuten, daß bei erhöhter Temperatur umgekehrt weniger Bakterien zur Ausfällung einer gegebenen Farbstoffmenge ausreichen werden. Tabelle III A gibt ein Bild von diesem umgekehrten Verhalten.

Die in Tab. III A verwendete Suspension von Cholera Bazillen war sehr konzentriert. Von dieser Ausgangssuspension waren die in der Tabelle angegebenen Verdünnungen hergestellt. Bei 0° wird die $\frac{1}{300}$ proz. Janusgrünlösung von einer $\frac{1}{100}$ Suspension ausgeflockt, während dazu bei 80° schon eine drei- oder viermal verdünntere genügt.

Agglutininbakterien¹⁾ verhalten sich, wie aus Tab. III B hervorgeht, genau wie normale; das Ausflockungsoptimum liegt für 0° annähernd bei $\frac{1}{600}$, für 20° bei $\frac{1}{300}$, für 40° bei $\frac{1}{150}$ Proz. Nachtblau.

¹⁾ Bechhold, Zeitschr. f. physik. Chemie 48, 385.

Tabelle III.

**A. Gleicher Farbstoff-
gehalt bei wechselnder
Bakterienmenge.**

**B. Farbstoff und
Agglutininbakterien.**

Cholera- suspension Ver- dünnungen Proz.	Janusgrün $\frac{1}{300}$ Proz.			Nachtblau End- verdünnung Proz.	Agglutinierte Colibakterien		
	0°	40°	80°		0°	20°	40°
$\frac{1}{10}$ bis $\frac{1}{50}$.	—	—	—	$\frac{1}{20}$ bis $\frac{1}{100}$.	—	—	—
$\frac{1}{100}$	+++	—	—	$\frac{1}{150}$	—	—	+++
$\frac{1}{250}$	—	+++	—	$\frac{1}{300}$	—	+++	—
$\frac{1}{300}$	—	—	+++	$\frac{1}{500}$	+++	—	—
$\frac{1}{400}$	—	—	+++	$\frac{1}{600}$ bis $\frac{1}{\infty}$.	—	—	—

Ferner macht es keinen Unterschied, wenn die Bakterien vorher auf 75° erhitzt oder selbst eine Stunde gekocht worden sind. Diese Behandlung pflegt das Optimum ein wenig zu verschieben, ändert aber nichts an der beobachteten Regel.

Tabelle IV. Nachtblaukonzentrationen, die zur optimalen Ausflockung von Bakterien bei verschiedenen Temperaturen erforderlich sind.

		Nachtblaukonzentrationen in Prozenten			
		Tem- peratur	Bazillen		
			nicht erhitzt	1 Stde. bei 75°	
Cholera bazillen	0°	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	Überall dieselbe Suspension
	20	$\frac{1}{100}$	$\frac{1}{140}$	$\frac{1}{100}$	
	40	$\frac{1}{100}$	$\frac{1}{120}$	$\frac{1}{100}$	
Coli comm. . .	0	$\frac{1}{200}$	$\frac{1}{200}$	$\frac{1}{200}$	Überall dieselbe Suspension
	20	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	
	40	$\frac{1}{140}$	$\frac{1}{140}$	$\frac{1}{140}$	
Pyocyaneus . .	0	$\frac{1}{240}$	$\frac{1}{200}$	$\frac{1}{240}$	Überall dieselbe Suspension
	20	$\frac{1}{100}$	$\frac{1}{240}$	$\frac{1}{100}$	
	40	$\frac{1}{140}$	$\frac{1}{160}$	$\frac{1}{140}$	
	60	$\frac{1}{120}$	$\frac{1}{140}$	$\frac{1}{140}$	

Es sei hier bemerkt, daß das Optimum nicht in jedem Experiment dasselbe ist. Es ändert sich vielmehr mit der Kon-

zentration der Bakteriensuspension, da es einerseits weniger Farbstoff bedarf, die Suspension auszuflocken, wenn sie verdünnter ist, da es andererseits unmöglich ist, Bakterienaufschwemmungen von genau gleichem Gehalt herzustellen. Allein die Proben der Tabelle IV sind vergleichbar, da für jede Bazillenart die gleiche Ausgangssuspension erhitzt, bzw. nicht erhitzt, zur Verwendung kam. Man bemerkt, daß das Optimum mit der Temperatursteigerung etwas ansteigt. Ähnliches wurde bei anderen Suspensionen und kolloidalen Lösungen beobachtet, wenn sie durch basische Farbstoffe ausgeflockt wurden.

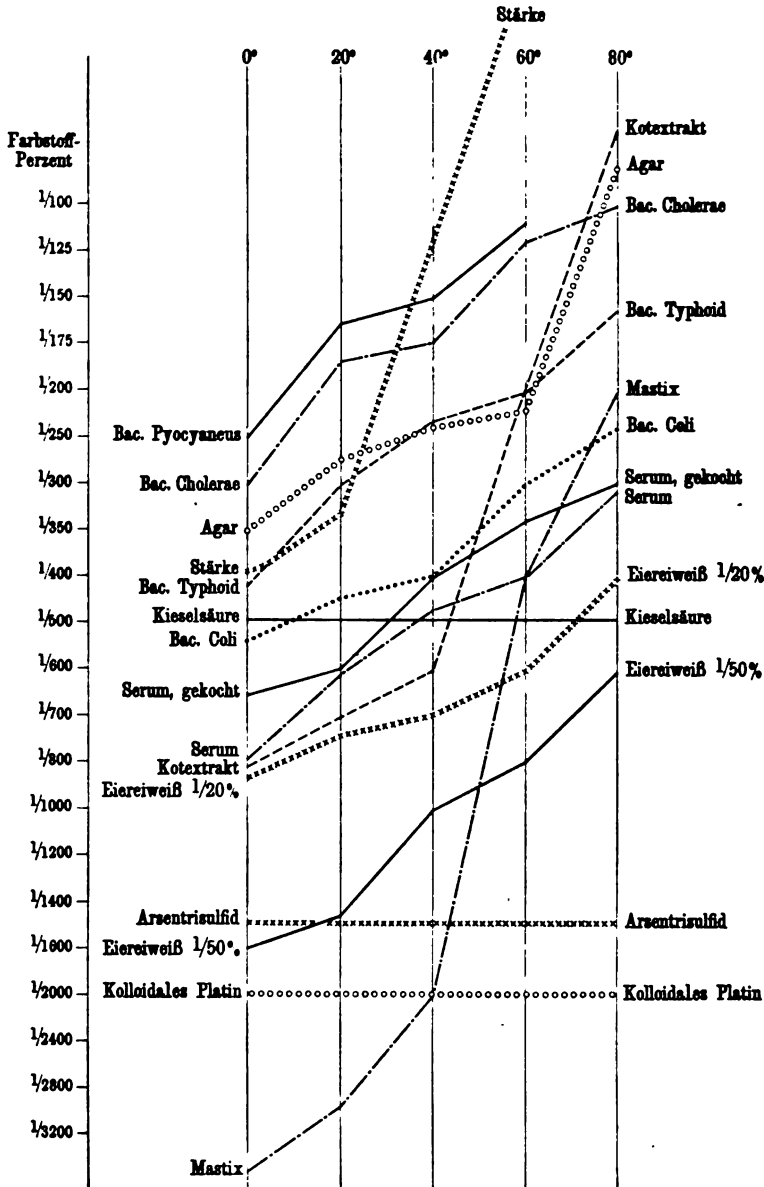
Tabelle V. Annähernde Ausflockungsoptima für Nachtblau und verschiedene negative Kolloide.

Kolloid	Verdünnung	Nachtblau, Prozente bei				
		0°	20°	40°	60°	80°
Stärke	dünne Lösung	1/400	1/350	1/150	keine Ausflockung	keine Ausflockung
Agar	1/80 Proz.	1/350	1/300	1/250	1/250	1/50 ¹⁾
Kotextrakt	1/400 der Ausgangslösung	1/800	1/700	1/600	1/200	1/50 ¹⁾
Mastix	1/60 der Ausgangslösung	1/4000	1/3000	1/2000	1/400 ¹⁾	1/200 ¹⁾
Kaninchenserum .	1/400	1/800	1/600	1/500	1/400	1/300
Kaninchenserum, gekocht	1/400	1/700	1/600	1/400	1/350	1/300
Eiweiß	1/50 Proz.	1/1800	1/1500	1/1000	1/800	1/600
Eiweiß, gekocht .	1/50 "	1/2000	1/1500	1/1500	1/1000	1/600
Gelatine	1/8 "	keine Flocken, partiell erstarrt	1/350	keine Flocken	keine Flocken	—

In Tabelle VI sei eine Versuchsreihe mit Mastix und Nachtblau ausführlich mitgeteilt. Obgleich sie ganz unabhängig von dem entsprechenden in Tabelle V angeführten Versuche ausgeführt war, stimmen die Ergebnisse innerhalb der Fehlergrenzen genau überein. Der umgekehrte Versuch, wo die Farbstoffkonzentration konstant bleibt und der Gehalt der Mastixemulsion wechselt, steht in Analogie mit dem Versuch in Tabelle III A, betreffend Cholera-bazillen und Janusgrün.

¹⁾ Unvollständige Flockenbildung.

Fig. 1.



Die vorstehende Kurventafel zeigt deutlicher als die Tabellen das regelmäßige Ansteigen der Mengen an Farbstoff, Nachtblau oder Janusgrün, die bei steigender Temperatur zur Aus-

Tabelle VI.

Nachtblau Proz.	Mastix, $\frac{1}{60}$ der Ausgangslösung					Mastix Verdünnung der Ausgangs- lösung Proz.	Nachtblau $\frac{1}{400}$ Proz.				
	0°	20°	40°	60°	80°		0°	20°	40°	60°	80°
$\frac{1}{20}$ bis $\frac{1}{100}$	—	—	—	—	—	$\frac{1}{4}$	—	—	—	—	—
$\frac{1}{200}$	—	—	—	—	+	$\frac{1}{6}$	+++	+++	++	—	—
$\frac{1}{250}$ bis $\frac{1}{400}$	—	—	—	—	—	$\frac{1}{10}$	—	—	+++	—	—
$\frac{1}{300}$	—	—	—	+	—	$\frac{1}{20}$	—	—	++	—	—
$\frac{1}{600}$ bis $\frac{1}{1000}$	—	—	—	—	—	$\frac{1}{30}$	—	—	—	—	—
$\frac{1}{1500}$	—	—	+++	—	—	$\frac{1}{60}$	—	—	—	+	—
$\frac{1}{2000}$	—	+	+++	—	—	$\frac{1}{80}$ bis $\frac{1}{450}$	—	—	—	—	—
$\frac{1}{3000}$	+++	+++	—	—	—	$\frac{1}{500}$	—	—	—	—	Sp.
$\frac{1}{4000}$	++	+	—	—	—	$\frac{1}{600}$ bis $\frac{1}{\infty}$	—	—	—	—	—
$\frac{1}{6000}$ bis $\frac{1}{\infty}$	—	—	—	—	—						

flockung einiger Kolloide erforderlich sind. Dabei fällt sofort auf, daß die aufsteigenden Linien — die der Bequemlichkeit wegen als Kurven bezeichnet sein mögen — einen doppelten Typus (I und II) aufweisen. Ein dritter Typus ist durch die Gerade vertreten. Die Lage der Kolloide in dem Kurvenbild ist ausschließlich durch die Konzentration bedingt. Die Resultate zweier Versuchsreihen mit Eiweiß sind nur mitgeteilt, um zu zeigen, daß gesteigerte Verdünnung bloß die Lage der Kurve, nicht aber ihren Typus ändert. Um ideale Kurven zu erhalten, müßte man die Konzentration der Kolloide so wählen, daß sie bei einem gegebenen Gehalt, z. B. $\frac{1}{1000}$ Proz. der Farbstofflösung, ihren Nullpunkt hätten. Das würde jedoch monatelange Arbeit erfordert haben, ohne den wirklichen Wert der bildlichen Darstellung zu erhöhen.

Von den Kurven zeigen einzelne zuerst ein allmähliches, dann bei höherer Temperatur ein sehr rasches; andere dagegen ein mehr gleichmäßiges Ansteigen. Der erstere Typus ist am deutlichsten bei Stärke, Kotextrakt und Mastix ausgesprochen. Nur bei Mastix findet sich am Schluß, bei 60 bis 80°, ein rapides Ansteigen. Der zweite Typus ist durch Serum, Eiweiß und Bakterien vertreten.

Wir finden, daß die Ausflockung der Kolloide des ersten Typus an Farbstoff bei 80° erfordert:

von Stärke	etwa ∞ mehr	als bei 0°
„ Mastix	20 mal mehr	„ „ „
„ Kotextrakt	16 „	„ „ „
„ Agar	$4\frac{1}{2}$ „	„ „ „

Ebenso bei den Kolloiden des zweiten Typus bei 80°

von Serum	etwa $2\frac{1}{2}$ mal mehr als bei 0°
„ gekochtem Serum	„ $2\frac{1}{2}$ „ „ „ „ „
„ Eiweiß $\frac{1}{30}$	„ 3 „ „ „ „ „
„ Eiweiß $\frac{1}{20}$	„ 2 „ „ „ „ „
„ Bac. coli comm.	„ $2\frac{1}{2}$ „ „ „ „ „
„ Bac. typhi abd.	„ $2\frac{1}{2}$ „ „ „ „ „
„ Bac. cholerae	„ 3 „ „ „ „ „

Agar scheint zunächst dem zweiten Typus anzugehören, doch entspricht seine Kurve zwischen 60 und 80° vielmehr dem Typus I.

Von den dem ersten Typus angehörenden Kolloiden sind Stärke und Agar reversibel und neigen daher bei Temperaturerhöhung zu einer Abnahme ihrer kolloidalen Beschaffenheit. Eine Mastixsuspension verliert beim Erhitzen viel von ihrer Undurchsichtigkeit, wird somit anscheinend weniger kolloidal. Vom Kotextrakt (klar filtrierter macerierter Darminhalt vom Kaninchen) ist nichts näheres bekannt; doch konnte die dunkelbraune Ausgangslösung, die keine Eiweißreaktion darbot, beliebig lange gekocht werden, ohne zu koagulieren, oder sonst eine merkliche Veränderung zu erfahren. Es scheint danach, daß sie ihre kolloidalen Eigenschaften einem reversiblen Kolloid verdankt, das beim Erhitzen minder kolloidal wird, vielleicht dem sogenannten Stercobilin, einem Umwandlungsprodukt der Gallenfarbstoffe.

Eine weitere Erscheinung, die dem Typus I anzugehören scheint, ist die unvollständige Ausflockung mit Agar, Kotextrakt und Mastix bis 80°, die das Bild der gegenseitigen Ausflockung von Kolloiden niederen Grades darbietet. Auch in diesem Punkte entspricht Agar mehr dem Typus I. Da Agar in einer Konzentration, die es zum Erstarren befähigt, nicht unter 100° schmilzt, so kann man nicht erwarten, daß seine kolloidale Beschaffenheit beim Erhitzen eher abnimmt als in der Nähe des Siedepunktes.

Dem Typus II gehören das Serum und Eiweiß an, die in verdünnter Lösung wahrscheinlich wenig durch eine Temperaturerhöhung bis 80° beeinflußt werden, oder, falls dies geschieht, eher zu einer Vergrößerung der Aggregate neigen dürften. Ihnen schließen sich die Bakterien an, die ja aller Wahrscheinlichkeit nach durch die Eiweißkörper ihrer Oberfläche, die als Schutzkolloid wirken, in Suspension erhalten werden.

Der dritte Typus, eine horizontale Gerade, findet sich bei den anorganischen Kolloiden: Arsentrisulfid, kolloidalem Platin und Kieselsäure. Sie bilden eine Gruppe für sich, die sich von den

organischen Kolloiden dadurch unterscheidet, daß zu ihrer Fällung bei erhöhter Temperatur nicht mehr Farbstoff erforderlich ist, als bei niederer.

In Tabelle VII sind zwei Versuche mit anorganischen Kolloiden näher ausgeführt.

Tabelle VII. Farbstoffe und anorganische Kolloide.

Janusgrün Proz.	Kolloidales Platin				
	0°	20°	40°	60°	80°
$\frac{1}{20}$ bis $\frac{1}{800}$	—	—	—	—	—
$\frac{1}{1000}$	+	+	+++	—	+++
$\frac{1}{1500}$	+++	+++	+++	+++	+++
$\frac{1}{2000}$	+++	+++	+++	+++	+++
$\frac{1}{3000}$	+	—	—	+++	+++
$\frac{1}{4000}$ bis $\frac{1}{\infty}$	—	—	—	—	—

Nachtblau Proz.	Arsentrisulfid				
	0°	20°	40°	60°	80°
$\frac{1}{20}$ bis $\frac{1}{1000}$	+	—	—	—	—
$\frac{1}{1500}$	+++	+++	+++	+++	+++
$\frac{1}{2000}$	+	+++	+++	+++	+++
$\frac{1}{3000}$	—	++	—	—	+++
$\frac{1}{4000}$	—	—	—	—	+++
$\frac{1}{5000}$ bis $\frac{1}{\infty}$	—	—	—	—	—

Tabelle VII zeigt, daß das Optimum der Ausflockung von kolloidalem Platin durch Janusgrün für alle Temperaturen, abgesehen von einer innerhalb der Fehlergrenzen liegenden Abweichung bei 40°, bei $\frac{1}{2000}$ Proz. liegt. Die Konzentration von $\frac{1}{1500}$ Proz. Nachtblau ist das Optimum der Ausflockung von Arsentrisulfid, wenngleich die Fällungszone bei 80° eine Verbreiterung zeigt. Es sei daran erinnert, daß der optimale Punkt nicht notwendig in der Mitte der Ausflockungszone liegt, sondern jener Konzentration entspricht, bei der die Flockenbildung am raschesten auftritt und in der ersten Zeit am meisten ausgesprochen ist. Ohne sorgfältige Beobachtung von Anfang an kann man ihn leicht übersehen.

Wird das Arsentrisulfid oder das kolloidale Platin vorher eine Stunde lang gekocht, so ändert das weder die Ausflockungszone, noch den optimalen Punkt. Das Kochen scheint auf diese zwei anorganischen Kolloide ganz ohne Einfluß zu sein.

Theoretische Betrachtungen.

Wir haben aus Tabelle III bereits entnommen, daß sich bei Variation des Bakteriengehalts und konstanter Farbstoffkonzentration eine absteigende Kurve ergibt. Ein ähnliches Experiment mit Mastix in Tabelle VI liefert eine umgekehrte Kurve. Die absteigende Kurve zeigt den gleichen Typus wie die aufsteigende Kurve, die durch Variation der Farbstoffkonzentration erhalten wird, so daß beide Kurven sich wie Spiegelbilder verhalten. Es ist daher einleuchtend, daß in jedem Fall mit dem Ansteigen der Temperatur mehr Farbstoff und weniger organisches Kolloid zur Auslösung der Flockenbildung benötigt werden. Bei den reversiblen Kolloiden sind die Unterschiede viel größer als bei irreversiblen.

Da Farbstoffe reversible Kolloide sind, so ist zu erwarten, daß sie in der Wärme weniger kolloidal werden, und Versuche mit Dialyse scheinen dies zu bestätigen. Hätten wir es sonach nur mit Typus II zu tun, wo der kolloidale Charakter der Bakterien sich anscheinend nicht ändert, während der des Farbstoffs mit Temperaturerhöhung abnimmt, so könnte die Erklärung der Erscheinung in der Annahme gefunden werden, daß der Farbstoff, indem er an kolloidaler Beschaffenheit verliert, auch an Fähigkeit einbüßt, die Bakterien auszuflocken, und umgekehrt die Bakterien in dem Maße fähiger werden, den Farbstoff auszuflocken, als dieser weniger kolloidal wird.

Eine solche Erklärung ist jedoch nicht stichhaltig, da Stärke, Agar und Mastix mit dem Ansteigen der Temperatur weniger kolloidal werden und daher auf die Kurven den umgekehrten Einfluß ausüben müßten. Aber im Gegenteil zeigen die Kurven mit diesen reversiblen Kolloiden bei höheren Temperaturen das steilste Ansteigen.

Wir können daher nur sagen, daß mit steigender Temperatur die Affinität der organischen Kolloide zu den Farbstoffen zunimmt, was durchaus mit den Erfahrungen der praktischen Färberei im Einklang steht, aber keine Erklärung der Erscheinung darstellt. Diese Regel trifft überdies für die anorganischen Kolloide nicht zu.

Es scheint schwierig, diese Temperaturkurven mit der geläufigen Theorie in Einklang zu bringen, wonach die Ausflockung durch die Neutralisation elektrischer Ladungen zustande kommt. Wenn die Annahme richtig ist, daß die Farbstofflösung durch Erwärmen weniger kolloidal wird, Serum und Bakteriensuspension aber nicht, dann wäre zu vermuten, daß in der Farbstofflösung die Zahl der elektrisch geladenen Aggregate zunimmt, und danach sollte weniger Farbstoff zur Ausflockung einer gegebenen Bakterienmenge erforderlich sein. Allein gerade das Gegenteil ist der Fall.

Es scheint kein genügender Grund für die Annahme gegeben, daß ein großes kolloidales Aggregat relativ oder absolut stärker geladen ist als ein kleineres. Wahrscheinlich besteht das Aggregat eines Farbsalzes aus einer Anzahl nicht dissoziierter Moleküle und einem dissoziierten, welchem es die elektrische Ladung verdankt. Bei Steigerung der Temperatur und entsprechend erhöhter Tendenz zur Ionisation dürften die Aggregate zerfallen und kleiner werden, aber jedes einzelne Aggregat dürfte noch ein dissoziiertes Molekül enthalten und die gleiche elektrische Ladung. — die natürliche Einheit — tragen, wie das größere Aggregat, aus dem es hervorgegangen ist.

Basische Hydroxyde.

Nimmt man statt eines basischen Farbstoffs ein basisches Hydroxyd, z. B. Eisenhydroxyd, so ergibt sich beim Erwärmen keine merkliche Änderung des Optimums.

In Tabelle VIII sind die Resultate angeführt, die einerseits mit zwei irreversiblen und zwei reversiblen Kolloiden, andererseits mit zwei negativen Farbsalzen erhalten wurden. An einigen Stellen ist eine Tendenz zur Erhöhung des Ausflockungsoptimums bei 80° erkennbar, doch sind wir geneigt, dies der Unvollkommenheit der Methode zuzuschreiben. Wenn kolloidales Ferrihydroxyd in einem Probierglas gekocht wird, so fällt es schließlich aus, vermutlich infolge der Spuren Kieselsäure, die aus dem Glas aufgenommen werden. Es ist sehr wohl möglich, daß Spuren von Kieselsäure — vermutlich kolloidaler — schon bei 80° in Lösung gehen, sich mit einem Teil des Ferrihydroxyds verbinden und so seine Wirkung abschwächen.

Ferrihydroxyd verhält sich sonach anscheinend gegenüber negativen Kolloiden sehr ähnlich wie Arsentrisulfid und die

Tabelle VIII. Ferrihydroxyd mit verschiedenen Kolloiden.

Fe(OH) ₃ Verdünnung	Serum 1/200 Proz.				
	0°	20°	40°	60°	80°
1/10 bis 1/20	—	—	—	—	...
1/40	+++	++	++	+	...
1/80	+++	+++	+++	+++	...
1/160	—	+	+++	+	...
1/200 bis 1/∞	—	—	—	—	...
Fe(OH) ₃	Stärke 1/8 Proz.				
1/10 bis 1/20	—	—	—	—	—
1/40	+++	+	+	+	+++
1/80	+++	+++	+++	+++	+++
1/160	+++	+++	+++	+++	—
1/200 bis 1/∞	—	—	—	—	—
Kongorot	Ferrihydroxyd 1/100 Proz.				
1/20 bis 1/200	—	—	—	—	—
1/400	+++	+++	+++	+++	+++
1/800	+++	+++	—?	+++	+++
1/1600	—	—	+++	—	+++
1/3200 bis 1/∞	—	—	—	—	—

Fe(OH) ₃ Verdünnung	Typhusbazillen				
	0°	20°	40°	60°	80°
1/50	—	—	—	—	—
1/100	—	—	—	—	+++
1/150	++	—	+++	+++	+++
1/200	+++	+++	+++	+++	—
1/250 bis 1/∞	—	—	—	—	—
1/20 bis 1/60	—	—	—	—	—
1/80	+++	++	+++	+++	+++
1/160	+++	+++	+++	+++	+++
1/320	+++	+++	+++	+++	+++
1/640 bis 1/∞	—	—	—	—	—
Fe(OH) ₃	Alizarinrot 1/400 Proz.				
1/20	—	—	—	—	—
1/50	—	—	—	—	+++
1/100	+++	+++	+++	+++	—
1/150 bis 1/∞	—	—	—	—	—

übrigen untersuchten anorganischen Kolloide gegenüber basischen. Bei graphischer Darstellung würde es eine Gerade geben.

Mastix scheint dagegen eine Ausnahme von dieser Regel zu bilden. Es gibt in diesem Falle eine Kurve, die dem Typus I der Tafel entspricht.

Tabelle IX. Ferrihydroxyd und Mastix.

Fe(OH) ₃ Verdünnung	Versuchsreihe I				
	Mastix $\frac{1}{60}$ Ausgangslösung				
	0°	20°	40°	60°	80°
$\frac{1}{20}$ bis $\frac{1}{200}$	—	—	—	—	—
$\frac{1}{400}$	—	—	—	—	+++
$\frac{1}{600}$	—	—	—	+	+
$\frac{1}{800}$	—	—	—	++	—
$\frac{1}{1000}$	—	—	+++	+	—
$\frac{1}{2000}$	—	—	+++	—	—
$\frac{1}{4000}$	+++	+++	+++	—	—
$\frac{1}{8000}$	+++	+	++	—	—

Mastix Verdünnung	Versuchsreihe II				
	Fe(OH) ₃ $\frac{1}{1200}$ Ausgangslösung				
	0°	20°	40°	60°	80°
$\frac{1}{2}$	+++	+++	+++	—	—
$\frac{1}{10}$	+++	+++	+++	—	—
$\frac{1}{20}$	+++	+++	+++	—	—
$\frac{1}{50}$	—	—	+++	—	—
$\frac{1}{100}$	—	—	—	+++	—
$\frac{1}{150}$	—	—	—	+++	+++
$\frac{1}{200}$	—	—	—	+++	+++
$\frac{1}{300}$	—	—	—	—	—

Tabelle IX zeigt, daß bei Erhöhung der Temperatur zur Ausflockung mehr Ferrihydroxyd und weniger Mastix benötigt wird. In der Versuchsreihe I ist bei 80° zehnmal mehr Ferrihydroxyd erforderlich als bei 0°, und ähnliche Verhältnisse weisen die anderen Proben auf. Die beiden Versuchsreihen wurden ganz unabhängig voneinander angestellt, trotzdem fallen die Optima sehr nahe zusammen. Bei den niederen Temperaturen sind die Zonen etwas breit und das Optimum konnte nicht sehr scharf bestimmt werden, aber bei 60 und 80° tritt es beiderseits sehr gut hervor.

So ergibt sich aus Reihe I bei 60° $1/800 : 1/60 = 1/1200 : 1/90$, während in Reihe II $1/100$ gefunden wird.

Aus Reihe I bei 80° berechnet sich $1/400 : 1/60 = 1/1200 : 1/180$, während der Versuch in Reihe II einen Wert zwischen $1/150 - 1/300$, annähernd $1/175$ ergibt.

Einige wenige Versuche mit Aluminiumhydroxyd lieferten ganz analoge Resultate wie jene mit Ferrihydroxyd, einschließlich der Ausnahme bei Mastix. Dieses wird sonach von den basischen Hydroxyden in sehr ähnlicher Weise beeinflusst wie die Farbstoffe; die anderen untersuchten Kolloide scheinen dagegen, wenn sie mit Hydroxyd zusammengehalten werden, einer ganz anderen Regel zu folgen.

Es scheint nicht möglich, für diese verschiedenen Erscheinungen eine allgemein gültige Deutung zu finden, doch scheinen die Versuche darauf hinzuweisen, daß wir es mit vier Gruppen von Kolloiden zu tun haben, zwischen denen, soweit es nach ihrem Verhalten gegen Wärmeschwankungen erkennbar ist, irgend welche tiefgreifende Unterschiede bestehen.

Kolloide	Bei Erhöhung der Temperatur
1. Farbstoffe	Leichter von organischen, nicht von anorganischen Kolloiden aufgenommen.
2. Reversible organ. Kolloide	Sehr gesteigerte Farbstoffaffinität, keine Steigerung gegenüber anorganischen Kolloiden ¹⁾ .
3. Irreversible organ. Kolloide	Mäßig gesteigerte Farbstoffaffinität, keine Steigerung gegenüber anorganischen Kolloiden ¹⁾ .
4. Anorganische Kolloide	Keine Änderung der Affinität für Farbstoffe oder andere Kolloide ¹⁾ .

Einige wenige Versuche mit basischen Hydroxyden gegenüber negativen anorganischen Kolloiden führten zu keinem positiven Ergebnis. Die Ausflockungszonen waren breit und die Optima konnten nicht befriedigend bestimmt werden. Immerhin waren Anzeichen dafür vorhanden, daß die Temperaturerhöhung keinen Einfluß auf die Lage des Ausflockungsoptimums hat.

Reversion.

Wie oben gezeigt, variiert die Zone der Ausflockung von negativen Kolloiden durch basische Farbstoffe erheblich mit der

¹⁾ Mit Ausnahme von Mastix gegenüber $\text{Fe}(\text{OH})_3$ und $\text{Al}(\text{OH})_3$.

Versuchstemperatur. Es schien von Interesse, festzustellen, ob nach Ausflockung bei einer gegebenen Temperatur mit Änderung derselben eine Wiederherstellung des ursprünglichen Zustandes eintritt. Tabelle X, Nachtblau — Stärke, ist typisch für die dabei gewöhnlich gefundenen Resultate. Sie ist ausführlich mitgeteilt, damit die Umkehrung in einer ganzen Reihe von Proben ersichtlich wird, was einen zufälligen Befund ausschließt.

Tabelle X. Reversion. Nachtblau und Stärke.

Nachtblau Proz.	Stärke $\frac{1}{8}$ Proz.			
	Versuchsreihe I		Versuchsreihe II	
	0° 24 Stdn.	24 Stdn. bei 0°, dann 24 Stdn. bei 40°	40° 24 Stdn.	24 Stdn. bei 40°, dann 24 Stdn. bei 0°
$\frac{1}{30}$ bis $\frac{1}{100}$. .	—	—	—	—
$\frac{1}{120}$	—	—	++	++ ¹⁾
$\frac{1}{140}$	—	+++	+++	+++ ¹⁾
$\frac{1}{160}$	—	+++	+++	+++ ¹⁾
$\frac{1}{180}$	—	+++	+++	+++ ¹⁾
$\frac{1}{200}$	—	+++	+++	+++ ¹⁾
$\frac{1}{240}$	—	+++	—	—
$\frac{1}{280}$	++	+++	—	— ²⁾
$\frac{1}{320}$	++	— ²⁾	—	— ²⁾
$\frac{1}{360}$	+++	— ²⁾	—	— ²⁾
$\frac{1}{400}$	+++	— ²⁾	—	— ²⁾
$\frac{1}{480}$	+++	— ²⁾	—	— ²⁾
$\frac{1}{560}$	++	— ²⁾	—	— ²⁾
$\frac{1}{640}$ bis $\frac{1}{\infty}$. .	—	—	—	—

Wie aus Tabelle X hervorgeht, ließen wir in Versuchsreihe I Nachtblau 24 Stunden bei 0° auf Stärke einwirken, die Ausflockungszone reicht von $\frac{1}{360}$ bis $\frac{1}{560}$ Proz. Nachtblau. Nachdem die Proben dann 24 Stunden bei 38 bis 40° gehalten worden waren, ergibt sich die Ausflockungszone von $\frac{1}{140}$ bis $\frac{1}{180}$ Proz. und die Proben $\frac{1}{320}$ bis $\frac{1}{560}$ Proz. zeigen völlige Umkehrung. Der Typus der Ausflockung entspricht praktisch genau jenen der ersten Spalten von Reihe II, wo vorher nicht mit einer Temperatur von 0° behandelt worden war.

¹⁾ Keine Umkehrung.

²⁾ Keine Flockenbildung bei 0°, wie sie ohne vorheriges Erhitzen auf 40° eingetreten wäre.

³⁾ Umkehrung.

In Reihe II, wo die Proben erst 24 Stunden bei 40°, dann die gleiche Zeit oder gar 48 Stunden bei 0° gehalten wurden, ist nicht bloß keine Umkehrung in den Proben mit $\frac{1}{120}$ bis $\frac{1}{330}$ Proz. nachweisbar, sondern es fehlt auch die Ausflockung von $\frac{1}{330}$ bis $\frac{1}{360}$, die ohne vorgängige Behandlung bei 40° hätte eintreten sollen.

Bakterien zeigen dasselbe Verhalten.

Tabelle XI. Reversion. Janusgrün und Cholera Bazillen.

Cholera- ausgangssuspension Verdünnung	Janusgrün $\frac{1}{200}$ Proz.				
	Normale Ausflockungszone			Änderung mit Temperaturerhöhung	
	0°	40°	80°	zuerst 0°, dann bei 40°	zuerst 0°, dann bei 80°
	24 Stdn.	24 Stdn.	3 Stdn.	24 Stdn.	3 Stdn.
$\frac{1}{10}$ bis $\frac{1}{40}$	—	—	—	—	—
$\frac{1}{50}$	++	—	—	— ¹⁾	—
$\frac{1}{100}$	+++	—	—	— ¹⁾	—
$\frac{1}{150}$	+++	+++	—	+++	— ¹⁾
$\frac{1}{200}$	+++	—	—	+	— ¹⁾
$\frac{1}{250}$	—	+++	—	+++	+ ²⁾
$\frac{1}{300}$	—	+++	—	+++	+ ²⁾
$\frac{1}{350}$	—	—	+++	—	+++
$\frac{1}{400}$	—	—	+++	—	+++

Cholera Bazillen			
Janusgrün Proz.	20° 24 Stdn.	80° 1 Stde.	1 Stde. bei 80°, dann bei 20° 24 Stdn.
$\frac{1}{20}$ bis $\frac{1}{60}$	—	—	—
$\frac{1}{80}$	—	+++	+++
$\frac{1}{100}$	—	+++	+++
$\frac{1}{120}$	—	+++	+++
$\frac{1}{140}$	+	++	++
$\frac{1}{160}$	+++	—	—
$\frac{1}{180}$	+++	—	—
$\frac{1}{200}$	+++	—	—
$\frac{1}{240}$	+	—	—

¹⁾ Völlige Umkehrung.

²⁾ Teilweise Umkehrung.

Die ersten drei Spalten der Tabelle XI geben die Ausflockungszonen der Cholerabazillen durch Janusgrün bei 0, 40 und 80°; Spalte 4 lehrt, daß eine vollständige Änderung im Ausflockungstypus eintritt, wenn die 0°-Reihe 24 Stunden bei 40° gehalten wird. Ebenso erfolgt die Ausflockung nahezu ganz nach dem Typus der 80°-Reihe, sobald die 0°-Reihe 3 Stunden auf 80° erwärmt worden ist. Wahrscheinlich wäre bei längerer Dauer des Erhitzens die Umkehrung auch bei $\frac{1}{250}$ und $\frac{1}{300}$ Proz. vollständig geworden. Nach Einwirkung von 80° wurden die Proben 24 Stunden bei 20° gehalten, ohne daß Änderung eintrat. Dieses Endstadium ist in der Tabelle nicht verzeichnet, wohl aber finden wir in Reihe 2, daß beim Heruntergehen von 80 auf 20° in den Proben mit $\frac{1}{80}$ bis $\frac{1}{140}$ Proz. Janusgrün keine Umkehrung eintritt, ebenso daß die Ausflockung bei $\frac{1}{160}$ bis $\frac{1}{240}$ Proz. ausbleibt, wo sie normalerweise bei 20° hätte erfolgen müssen.

Andere untersuchte Bakterien, so Typhus-, Coli- und *Pyocyanus*-bazillen, zeigen die gleiche Reversion sowohl mit Janusgrün als auch mit Nachtblau.

Eine Umkehrung vom Typus der höheren zu jenem niedrigerer Temperatur wurde niemals beobachtet, aber auch die Reversion von niedrigen zu hohen Temperaturen gibt nicht bei allen Kolloiden befriedigende Resultate. Die Ausflockungszonen von Serum- und Eiereiweiß sind breit und decken sich bei verschiedenen Temperaturen zum Teil, so daß die Randzonen, in denen sich die Umkehrung zeigen kann, verhältnismäßig schmal sind, so daß ihr Auftreten einigermaßen zweifelhaft bleibt. Doch sind Anzeichen dafür vorhanden, daß Serumalbumin eine unvollkommene Umkehrung zeigt, Eialbumin aber nicht.

Es ist von Interesse, daß Bakterien, die an sich leicht die Reversion der Ausflockung zeigen, sich nach einstündigem Kochen insofern abweichend verhalten, als sie keine Umkehrung der durch Erwärmen erreichten Ausflockung, aber auch keine Behinderung derselben bei niedriger Temperatur zeigen, wie sie sonst nach Erwärmen eintritt.

Nach dem Erhitzen der Bakterien auf 75° ist die Reversion vielleicht etwas weniger deutlich als vorher ohne vorgängiges Erhitzen. Bei der großen Zahl der sowohl mit unerhitzten als mit gekochten Bakterien ausgeführten Versuche kann an der Richtigkeit der Beobachtung kein Zweifel sein.

Aus Tabelle XII entnimmt man, daß die Ausflockung von gekochten Bakterien durch Janusgrün bei 0° von $\frac{1}{300}$ bis $\frac{1}{400}$ Proz.

Tabelle XII. Reversionsversuch. Gekochte Typhusbazillen und Janusgrün.

Die Typhusbazillen in dünner Suspension.

Janusgrün Proz.	Erst niedrige, dann höhere Temperatur					
	0°	erst 0°, dann 40°	40°	20°	erst 20°, dann 5 Stdn. bei 60°	3 Stdn. bei 60°
1/20 bis 1/60 . .	—	—	—	—	—	—
1/100	—	—	—	—	—	—
1/150	—	—	—	—	++	+
1/200	—	—	—	—	+++	++
1/250	—	+++	+++	—	+++	+++
1/300	+	+++ ¹⁾	—	+++	+++ ¹⁾	—
1/350	++	+++ ¹⁾	—	++	++ ¹⁾	—
1/400	++	++ ¹⁾	—	++	++ ¹⁾	—
1/500 bis 1/∞ .	—	—	—	—	—	—

Janusgrün Proz.	Erst höhere, dann niedrige Temperatur					
	2 Stdn. bei 80°	80°, dann 20°	20°	40°	40°, dann 0°	0°
1/20 bis 1/60 . .	—	—	—	—	—	—
1/100	+	+	—	—	—	—
1/150	++	+++	—	—	—	—
1/200	+++	+++	—	—	—	—
1/250	—	—	—	+++	+++	—
1/300	—	— ²⁾	+++	—	— ²⁾	+
1/350	—	— ²⁾	++	—	— ²⁾	++
1/400	—	— ²⁾	++	—	— ²⁾	++
1/500 bis 1/∞ .	—	—	—	—	—	—

erfolgt, und daß nach 24stündigem Verweilen bei 40° keine Reversion eintritt, sondern der 40°-Typus sich einfach dem 0°-Typus superponiert, so daß nun die Ausflockung von 1/250 bis 1/400 Proz. reicht. Die gleiche Erscheinung ist bei Erhöhung der Temperatur von 20 auf 60° zu beobachten. Beim Herabgehen der Temperatur von 80 auf 20° oder von 40 auf 0° tritt keine Änderung ein.

¹⁾ Hier wäre Umkehrung eingetreten, wenn die Bakterien nicht gekocht worden wären.

²⁾ Hier wäre Ausflockung eingetreten, wenn nicht die Behandlung bei höherer Temperatur vorangegangen wäre.

Erklärungsversuche für diese Erscheinung.

Nach dem Mitgeteilten wird der Typus der Ausflockung negativer organischer Kolloide durch Farbstoffe beim Ansteigen der Temperatur z. B. von 0 auf 40° durchaus verändert, während das Absinken z. B. von 40 auf 0° ohne Einfluß ist. In der Ausflockungszone besteht offenbar eine Bindung zwischen beiden Kolloiden. Werden die beiden entgegengesetzt geladenen Kolloide in einem solchen Verhältnis gemischt, daß keine Ausflockung eintritt, und nun ein elektrischer Strom durchgeschickt, so wandert das im Überschuß vorhandene Kolloid nach seiner Elektrode und führt das in geringer Menge vorhandene mit sich. Beide Kolloide müssen sonach miteinander verbunden sein, wenngleich sonst kein Anzeichen dafür gegeben ist.

Nehmen wir weiter beispielshalber an, daß es sich um einen Eiweißkörper handelt, der sich gegen Temperatursteigerung so verhält, wie Bakterien gegen Nachtblau, und nehmen weiter an, daß die Aggregate des Eiweißkörpers beim Hinaufgehen der Temperatur von 0° auf 40° nicht verändert werden, hingegen die Aggregate der Nachtblaulösung zerfallen und kleiner werden, so ist klar, daß, falls jedes Eiweißaggregat bei 0° eine bestimmte Zahl, z. B. zwei Nachtblauaggregate absorbierte, es bei 40° mehr, z. B. vier solche Aggregate absorbieren wird. Nun mögen vier solche kleineren Nachtblauaggregate nicht zur Ausflockung ausreichen, dazu mögen, da das Optimum bei 8 liegt, 6 bis 12 erforderlich sein. Entsprechend daher das Optimum der Ausflockung bei 0° einer $\frac{1}{300}$ Proz. Nachtblaulösung, so wird bei 40° eine doppelt so hohe Konzentration, $\frac{1}{100}$ Proz., erforderlich sein¹⁾.

Wenn nach der Ausflockung bei 0° die Temperatur auf 40° erhöht wird, so besteht kein Hindernis, daß sich von der freiliegenden Oberfläche der Nachtblauaggregate kleinere Aggregate loslösen; die so entstandenen Tochteraggregate verteilen sich auf die Eiweißaggregate unter Veränderung des Ausflockungstypus zu dem für 40° geltenden, d. h. es kommt zu Zerfall der Flocken, da bei $\frac{1}{300}$ Proz. bloß vier Farbstoffaggregate auf jedes Eiweißaggregat kommen. Bei $\frac{1}{100}$ Proz. aber wird Ausflockung eintreten, weil dann je acht auf ein Eiweißaggregat einwirken können. Beim Herabgehen der Temperatur von 40 auf 0° bleiben die acht

¹⁾ Wir verkennen nicht, daß bei Steigerung der Konzentration eine Neigung zur Vergrößerung der Aggregate besteht, so daß das hypothetische Optimum bei $\frac{1}{150}$ Proz. statt $\frac{1}{100}$ liegen könnte. Doch war es bei obiger Darlegung nicht notwendig, auch diesen Punkt in Rechnung zu ziehen

an jedem Eiweißaggregat anhaftenden Farbstoffaggregate einzeln an dieses gebunden und sind daher unfähig, größere Aggregate unter sich zu bilden. Daher bleibt der Typus der Ausflockung unverändert und geht nicht in den 0°-Typus über.

Nach dieser Auffassung könnte sich der Ausflockungstypus bei Temperatursteigerung ändern, ohne daß ein Zerfall der Verbindung beider Kolloide vorauszugehen brauchte, während bei Temperaturabnahme erst ein Zerfall der schon vorhandenen Verbindung und eine neuerliche Bindung von etwas anderem Charakter erfolgen müßte, damit sich der Ausflockungstypus ändert.

Es bliebe noch die Superposition des 40°-Typus über den 0°-Typus zu besprechen, wie sie bei gekochten Bakterien und wahrscheinlich auch beim Eiereiweiß als Folge der Temperatursteigerung zu beobachten ist; doch möchten wir zunächst auf eine Deutung dieser Erscheinung verzichten.

Diese Versuche über Reversion weisen anscheinend darauf hin, daß in der Färberei eine bessere Fixation und Waschbeständigkeit erreicht wird, wenn das Färben bei höherer Temperatur ausgeführt wird. Tatsächlich wird das Färben, abgesehen von dem Fall, daß es sich um eine Diazotierung auf der Faser handelt, bei erhöhter Temperatur ausgeführt, um die Aufnahme des Farbstoffs zu beschleunigen, und wir wissen nicht, ob dabei jemals die Fixation in Betracht gezogen worden ist. Wir haben in den gebräuchlichen Lehrbüchern über Färberei nach einem sicheren Hinweis gesucht und nur folgende wichtige Bemerkung von Georgiewicz¹⁾ gefunden, die sich auf die Wirkung basischer Farbstoffe, auf mit Farbsäure oder Fettsäuren gebeizte Baumwollfaser bezieht: „Manche Färber ziehen es vor, ohne Erwärmen zu färben, da so glänzendere Farben erhalten werden; doch sind diese nicht so gut fixiert, wie bei Anwendung des Wärmeverfahrens (60°) und die Methode ist daher nur für hellere und mittlere Nuancen zu empfehlen.“

In den angeführten Versuchen wurde die Temperatur während der ganzen Beobachtungszeit konstant erhalten: die Tatsache, daß das Herabgehen der Temperatur keine Änderung des Ausflockungstypus bedingt, weist darauf hin, daß es zur Erreichung des Wärmetypus genügen dürfte, die zwei Kolloide bei der betreffenden Temperatur, 60 bis 80°, zu mischen, die Proben nach einigen Minuten aus dem Wasserbade herauszunehmen und bei 20°

¹⁾ Chemical technology of textile fibres, p. 145.

sich selbst zu überlassen. Tabelle XIII spricht für die Richtigkeit dieser Schlußfolgerung, doch reicht die Zahl der Versuche nicht zur Sicherstellung dieses Punktes aus.

Tabelle XIII. Colibazillen.

Nachtblau Proz.	Kontrollversuche		Gemischt bei 60°, in 5 Min. auf 20° abgekühlt, 24 Stdn. Stehen	Janusgrün Proz.	Kontrollversuche		Gemischt bei 60°, in 5 Min. ab- gekühlt auf 20°, 24 Stdn. Stehen
	20° 24 Stdn.	60° 2 Stdn.			20° 24 Stdn.	60° 2 Stdn.	
1/50 . . .	—	—	—	1/50 . . .	—	—	—
1/100 . .	—	++	+++	1/100 . .	—	+++	+++
1/150 . .	+++	—	—	1/150 . .	—	—	—
1/200 . .	+++	—	—	1/200 . .	+++	—	—
1/250 . .	—	—	—	1/250 . .	+++	—	—

Man darf aus der Tabelle schließen, daß die Verbindung der zwei Kolloide, wenigstens in der Wärme, fast augenblicklich erfolgt, die eigentliche Ausflockung aber langsamer zustande kommt.

Schlußfolgerungen.

1. Ausflockung durch Farbstoffe.

- Zur Ausflockung negativer organischer Kolloide bedarf es um so mehr Farbstoff, je höher die Temperatur ist.
- Der progressive Mehrbedarf an Farbstoff bei Temperaturerhöhung ist bei reversiblen Kolloiden viel größer als bei irreversiblen.
- Bei anorganischen Kolloiden fehlt er.

2. Ausflockung durch basische Hydroxyde.

Die Menge an basischem Hydroxyd, die nötig ist zur Ausflockung negativer organischer Kolloide, ist von der Temperatur unabhängig. Mastix bildet anscheinend eine Ausnahme.

3. Reversion der Ausflockung von organischen Kolloiden durch Farbstoffe.

- Bei Erhöhung der Temperatur kann der für eine niedrigere Temperatur geltende Ausflockungstypus gänzlich in den für eine höhere Temperatur geltenden übergeführt werden.
- Erniedrigung der Temperatur ändert den Ausflockungstypus nicht.

THE TREATMENT OF THYROIDISM BY A SPECIFIC CYTOTOXIC SERUM.*

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During the past two years a large number of cases of hyperthyroidism have been treated with a specific serum.¹ Preliminary papers² have been published dealing with the preparation of the serum and the therapeutic results obtained by its use. In the present paper we propose to discuss the questions arising from this study from the standpoint of accumulated experience and it is hoped to make the method so plain that any physician may understand the rationale of the treatment and the special points to be observed in its application. There are two fundamental propositions to be considered, viz., the nature of the serum and the nature of the disease. These will be considered in order.

THE SERUM.

We have called the serum which we have used specific because we believe that it has a special action on the thyroid gland, and it is necessary for the argument that the grounds for such belief should be reviewed. It is now a well-known and universally admitted fact that the proteid of each animal species has a characteristic physicochemical structure; in other words, it is specific. The chemical methods by which differences in structure are determined have not as yet been refined to a sufficient extent to render it possible to make a chemical distinction between proteids from closely related species of animals, but the methods employed in immunity investigations of the last few years have been thus refined and their reliability has been demonstrated in such degree that the results obtained by these methods are accepted by most investigators as demonstrating specific characters of the proteid in each animal species. This specificity is not absolute, for it has been found that a

*This article represents the Mütter Lecture which was delivered before the College of Physicians in Philadelphia, Dec. 13, 1907.

1. It will be recalled that the serum to which we refer is made by inoculating animals (rabbits or sheep), with the pure proteids from the human thyroid gland. In the beginning the proteids were obtained only from goitrous glands, but more recently the proteids from normal glands have been used successfully.

2. Beebe, S. P.: Preparation of a Serum for the Treatment of Exophthalmic Goiter, *Jour. Am. Med. Assn.*, 1906, xlv, 487. Beebe: *Tr. Assn. Am. Phys.*, 1906, p. 548. Rogers, John: The Treatment of Thyroidism by a Specific Serum, *Jour. Am. Med. Assn.*, 1906, xlvii, 655.

serum made against the proteid of a given species will show a mild reaction against the serum from closely related species, but by observing the time and the dilution in which the reaction takes place it is possible to distinguish sharply between species. Heterologous proteids, that is, proteids from different species, may be readily separated by means of these serum reactions, and it seemed to us reasonable to believe that a method capable of making such fine distinctions would enable one to differentiate between homologous proteids, that is, between proteids taken from the different organs of the same species. The widely varying functions of the different organs are dependent on the physicochemical structure of the various cells which compose them, and it would seem there must exist sufficient individuality of structure in organs which have such widely varying functions as the liver and kidney to permit of its recognition by the highly selective biologic method. One of the most suggestive researches in this line was that of Uhlenhuth,³ who found it possible to differentiate sharply between the proteids in the white and yolk of the egg by means of a serum made against one or the other of these proteids. He found that it was possible to get a mild reaction to the proteids of the yolk by means of serum developed against the proteids of the white of the egg, but only slowly, even in high concentrations of serum, so that in point of time and in delicacy the reactions of these homologous proteids against their own serum was specific.

If the serum is developed by injecting the pulp of an organ into some alien species of animal, we shall develop anti-bodies against all the proteids so introduced, and it is obvious that such a serum must contain a number of factors. Certain of these may be specific, since they are developed against the proteid which is found only in the particular organ in question, while others, such as those developed against the blood, must be common. A very large number of experiments are now on record which support such a conclusion, but in many of them the specific action is so obscured by the common action that its demonstration has not always been satisfactory. In a paper published three years ago by one of us⁴ this question was discussed and the reasons were given for choosing certain pure proteids—viz., the nucleoproteids—from organs as nearly blood-free as practicable, for developing specific antiserum. Reasons were given in that paper for believing that relatively more of

3. Uhlenhuth: *Zur Lehre von der Unterscheidung verschiedener Eiweissarten mit Hilfe specifischen Sera*. Festschrift, Geburtstag von Rob. Koch, Jena, 1903. p. 49.

4. Beebe, S. P.: *Cytotoxic Serum Produced by the Injection of Nucleoproteids*, Jour. Exper. Med., vii, 733.

the specific factors and less of the common element were produced than by including the whole organ pulp in the injected material. The specific action of such serum has been determined by the precipitin test, the agglutinin test, and by injection of the serum *in vivo*. The precipitin test is well known. The agglutinin test is similar to that employed in bacterial reactions, except that a suspension of finely divided tissue fragments is substituted for the bacterial emulsion. Such reactions *in vitro* show that homologous proteids may be differentiated sharply from one another. In point of time and in completeness of the reaction the serum is specific. When introduced into the body the reactions are specific in the sense that severe lesions can be produced in the selected organ without causing a corresponding lesion in other organs.

The serum has at least two factors: first, the factor common to proteids of all the organs of the body, and to a lesser degree the proteids of closely related species; second, the specific factor acting on one particular organ. We have found it possible to demonstrate these two factors by means of absorption experiments. If we inject dog-kidney nucleoproteids into a rabbit we produce a serum which gives a mild reaction with dog serum, also with proteids from the dog's liver or other organs, but a very marked reaction with kidney proteids. If we now add to this serum a considerable quantity of dog-liver tissue ground to fine hash and allow the two to stand in contact for a few hours in the refrigerator, and then filter, first through paper, and later through a Berkefeld candle, we find that the common factor of the serum has been removed.⁵ Such serum no longer has any precipitating or agglutinating effect on liver proteids or suspension of liver tissue fragments, nor does it act on any other proteids except those from the kidneys, and on the latter its action, although very marked, is somewhat less than before the absorption. By the addition of a large number of washed erythrocytes, one may also absorb the common factor. Such absorbed serum will cause kidney lesions when injected intravenously into the dog's circulation. If, however, one absorbs the kidney serum with kidney tissues one finds that the specific factors and the common factors have both been removed.⁶

The serum which has just been described was made from organs containing a small amount of blood, and one of the criticisms which has been made of this work is based on the fact that the serum itself is slightly hemagglutinative and hemolytic. The lesions found in the body after the injection of such serum are explained by these critics (Pearce

5. The liver tissues used for this purpose were not blood-free.

6. Beebe, S. P.: Nucleoprotein Immunity, Brit. Med. Jour., 1906, p. 1786.

and Jackson⁷) by assuming that the serum has caused the formation of small emboli of erythrocytes and that the resulting thrombi have caused anemic necroses. The lesions which we found, however, were not of that type, and moreover, they were produced also by serum from which hemolytic factors had first been absorbed in the manner described above; we conclude, therefore, that they have been produced by a special organ cytotoxin. We have some corroborative evidence that the hemolysins are not actively concerned in producing the lesions, viz., the plain unabsorbed serum is slightly hemolytic *in vitro*, and yet when injected into an animal it invariably was followed in a few hours by the presence of an increased number of red cells in the blood and at the same time a severe lesion was being produced in some specific organ. We have in such findings no evidence of blood destruction.

We have reviewed the principles of the serum at this length because it is of fundamental importance that we establish reasons for believing that serum made from the thyroid gland has a special action on that gland; for obviously if the serum acts as much on the kidneys, the liver, or the intestinal epithelium as it does on the thyroid, we have no logical ground or excuse for using it in the treatment of disease of the thyroid gland.

The methods followed in the making of the serum have been described in a previous article¹ and no essential change has been made during the last year.⁸ We are not yet fully acquainted with the differences between

7. Pearce and Jackson: Concerning the Production of Cytotoxic Sera by the Injection of Nucleoproteids, Jour. Infect. Dis., 1906, iii, p. 742.

8. The various steps in the production of this serum are briefly as follows: The fresh, human thyroid glands, which must not be preserved in formalin, or alcohol, or any substance which coagulates the albumin, are ground to a very fine pulp and extracted in several times their volume of salt solution made faintly alkaline with sodium hydroxid. Extraction is carried on in a refrigerator for twenty-four to thirty-six hours, and chloroform or thymol may be added to prevent bacterial growth. The coarser particles in the extract are then removed by straining through gauze, the filtrate centrifugated and filtered through paper. A clear extract may be readily obtained by filtering the strained extract through a Buchner funnel filled with paper pulp. We have found that a very convenient method for precipitating the proteids from this extract is to acidify with acetic acid to a concentration of 0.1 per cent., add a sufficient amount of saturated sodium chlorid solution to make the concentration of the salt in the extract 10 per cent. of saturation, and then heat to 44 degrees C. for half an hour. Under these conditions we obtain an abundant flocculent precipitate which may be readily washed, redissolved by the addition of a small amount of alkali and reprecipitated by the addition of acetic acid. The proteid is redissolved and reprecipitated three times, and is thoroughly washed each time. The purified proteid is dissolved in alkaline salt solution for inoculation into the peritoneal cavity of the rabbit or sheep in which we develop the serum. From five to eight inoculations one week apart are given to each animal before the blood is withdrawn and the serum prepared in the usual method.

a serum made from the nucleoproteid and that from the nucleoproteid and globulin combined; but we are still inclined to the belief that the serum from the nucleoproteid is more cytotoxic than that from the globulin, though the latter proteid is more efficient in the development of antitoxins.

We wish again to emphasize the fact that the serum must be made from human thyroid glands, because of the biologic specificity of proteids. It would be quite as logical to use tetanus antitoxin in the treatment of diphtheria as to use a serum made against sheep thyroid in the treatment of a human subject. We can get no evidence *in vitro* that such a serum has any antagonistic action against the human thyroid proteids. On this ground alone, therefore, the negative results which Murray⁹ obtained in the use of serum made in a goat against sheep proteids are precisely what should have been expected. Our serum has been made in sheep and rabbits against the proteids of the human thyroid gland.

One never reaches a condition of immunity at all comparable with the immunity to a diffusible bacterial toxin. From three to four times the initial dose of proteid may be safely given at the end of the immunization period, but one can not go beyond this point.

Although the serum shows a high degree of antagonistic action *in vitro* it does not in the immunized animal protect against excessive doses of the specific proteid. We have, nevertheless, data from some experiments which indicate that it does have some power of conferring passive immunity. Six fresh adult rabbits were kept under observation for some days to make sure that they were in a healthy condition. One group of three was then given for two successive days 5 c.c. doses of an active thyroid antiserum previously made in other rabbits. There was no reaction because of the biologic identity of the serum. Twelve hours following the second injection of serum, all six rabbits were given large doses of human thyroid proteids. The three protected rabbits had a milder immediate reaction and the late effects, such as loss of weight, were much less marked in this group than in the non-protected group. Clinically, we see acute toxic cases in which the subjects experience very marked and quite immediate beneficial results from the injection of the antiserum, a result quite similar to what we should expect if we were neutralizing a toxic substance. Obviously we can not be so certain of our ground here as we can in a laboratory experiment in which we can control matters so as to have fewer unknown factors.

9. Murray: *Lancet*, London, Nov. 11, 1905.

THE DISEASE.

In our previous paper¹⁰ we have outlined the evidence for believing the symptoms of the disease to have their origin in a condition of hyperactivity of the thyroid gland. Such a mode of origin is a fundamental premise in our treatment of these cases with an active antiserum designed to neutralize the toxic effects of thyroid proteid in the circulation, and also to inhibit the secretory activities of the gland. The symptoms of thyroid cases, tremor, nervous irritability, tachycardia, diarrhea, perspiration, rapid loss of weight, have all been produced in animals and also in the human subject by the administration of thyroid preparations. The almost constant occurrence of goiter, the histologic appearances indicative of increased activity, and the greatly increased circulation through the gland vessels, together with the amelioration of the condition by surgical removal, are additional arguments that the gland is a fundamental factor in the disease. The large relative lymphocytosis, and the diminished excretion of kreatinin with increased output of kreatin are the usual findings in the typical well-developed patient with Basedow's disease, and these blood findings have likewise been produced by the administration of thyroid preparations to healthy animals.¹¹ A discussion of the etiology of the disease is not within the scope of the present paper, and we accept provisionally the theory that in typical cases the characteristic symptoms have their origin mainly in the hypersecretion from the thyroid gland.

There are certain limitations to our knowledge in this matter, however, which should be pointed out. It is not known whether the physiologic activity of the gland is dependent solely on the iodized proteid therein contained, and, if so, whether the activity is measured by the quantitative amount of iodine present. As has been pointed out elsewhere, there is an increased amount of nucleoproteid¹² in the gland in Graves' disease, but this is a function of the cellular hyperplasia and we do not know whether or not it has any significance in the physiologic action of the secretion.

There is no experimental evidence to determine whether or not the secretion from the gland in Graves' disease has more or less physiologic activity than the normal gland or whether the secretion is so altered in character as to have an entirely different sort of action, and in the dis-

10. Beebe, S. P.: Tr. Assn. Am. Phys., 1906, p. 548.

11. Perry: Some Studies of the Blood in Thyroid Feeding in Insanity, Med. Rec., August, 1906.

12. Beebe, S. P.: Physiology of the Thyroid Gland in Its Relation to Exophthalmic Goiter, Jour. Am. Med. Assn., Oct. 5, 1907, xlix, 1155.

eased condition we must consider the possibility of a defective functioning of other organs which normally are stimulated by the secretion. We have very few methods of estimating the physiologic value of the secretion under normal conditions, the measurement of the protective power to acetonitril poisoning proposed by Reid Hunt¹³ being the only one having quantitative possibilities.¹⁴ Until the normal physiology of the gland is better understood we can only deal with the surface indications; and these point to the thyroid gland as one important source of the symptoms in typical cases of hyperthyroidism.

Graves' disease presents a great variety of clinical pictures which in many instances vary so widely from the typical text-book description as to be scarcely recognizable. It has been observed for many years that there is often a progression from a typical Graves' disease through a mixed, indefinite type to a characteristic condition of myxedema. These clinical conditions are the accompaniment of changes in the pathologic structure of the thyroid gland.

Ewing¹⁵ has described four distinct types or stages in the natural history of the thyroid gland of Graves' disease:

1. In the early stages of the disorder, only hyperemia with increase of colloid showing diminished staining reaction with eosin. Microscopically the gland may show no gross changes.
2. Hyperemia with increased colloid, and cellular hyperplasia.
3. Extensive cellular hyperplasia, with increase of imperfect alveoli lined by large or giant cells, and nearly complete loss of colloid.
4. Late stages with fibrosis, atrophy of cells, sclerosis of vessels, hemorrhages and cysts.

There is thus a complete series of changes from the thyroid of early Graves' disease to the pronounced alterations of myxedema, with many intermediate forms, especially in the older cases. It is obvious that the symptoms accompanying changes of such diversity must vary extremely and it is not to be expected that any one treatment, however effective it may be in one stage, can be equally applicable to all stages or types of the disease. Moreover, as the disease progresses, complications may arise in other organs, as, for example, chronic myocarditis, so that the therapeutic problem in advanced cases becomes extremely complex. We

13. Hunt, Reid: Influence of Thyroid Feeding on Poisoning by Acetonitril, *Jour. Biol. Chem.*, 1905, i, p. 33.

14. We have made a series of experiments on this point which will be reported later in detail, but our conclusion is that the method is not suitable for detecting small quantitative differences.

15. Ewing: Pathology of Exophthalmic Goiter in Its Relation to the Serum Treatment, *New York Med. Jour.*, 1906, p. 1061.

do not know how important the relations of the thyroid to other ductless glands may be. The thymus gland has been found hypertrophied in those cases in which autopsies have been made in New York during the last few years, and the lymphatic tissue in general increased in amount. Hansemann¹⁶ is of the opinion that these changes are secondary to the functional overactivity of the thyroid. These facts must be correlated with the high lymphocytosis characteristic of all severe cases and with the fact that it is possible to produce a lymphocytosis by the administration of thyroid to normal animals. It appears, then, that in the young subjects with characteristic symptoms of a recently developed condition of hyperthyroidism we are dealing with a comparatively specific disease which may be alleviated by neutralizing the toxic secretion of the gland; but in the older cases which have existed so long as to cause secondary changes and which may have symptoms of both Graves' disease and myxedema, or which indeed may be a myxedema in nearly every particular, masquerading under the name of Graves' disease because of the primary condition of the patient, it is not to be expected that the serum will have curative effect. And such a conclusion must apply with equal force to the operative removal of the gland or to any form of treatment aimed directly at the thyroid. Analogously, if diphtheria were a chronic disease extending over some months, one would not expect to restore the damaged heart, kidney and nervous system in an advanced case by any amount of serum prepared against the Klebs-Loeffler bacillus and its toxins. There is an abundance of clinical evidence demonstrating the specific action of thyroid preparations in myxedema, and in a small percentage of cases of Graves' disease improvement is obtained by careful thyroid treatment, but by far the majority of typical cases of this disease are made worse by such medication. It is therefore of prime importance to recognize the type of the particular case in order to treat it intelligently. We emphasize this point, because in some instances cases of myxedematous type have been treated by serum, and occasionally, indeed, by operation with very unsatisfactory results.

CLASSIFICATION.

It is impossible to make a comprehensive classification of patients suffering from thyroid disease unless one makes almost as many groups as there are patients. In determining whether a given case is a favorable one for serum treatment it is necessary to study the condition carefully to determine if possible whether the symptoms are those of hyperthyroidism or hypothyroidism. A careful study of the blood, including

16. Hansemann: Berl. klin. Wehnschr., Oct. 30, 1905.

the number of erythrocytes, the percentage of hemoglobin, and a differential leucocyte count, together with a quantitative analysis of the nitrogenous constituents of the urine, will be of value in this respect. In the majority of cases it is possible to decide this point, but we have not found any criterion accurate in all cases and we suggest the necessity for thorough study of the doubtful types in order to establish a more reliable means of classification. The following classification is made solely for the purpose of serum treatment, with a full recognition of the fact that it does not cover all cases, and with the reservation that it is necessary to experiment carefully with many of the atypical cases before one can determine what form of treatment is most suitable. In making these groups we take into consideration the age of the patient, the probable condition of the thyroid, the clinical type of the disease as regards symptoms referable to the thyroid, and also as regards secondary changes in other organs.

TYPES FAVORABLE FOR SERUM TREATMENT.

1. Typical exophthalmic goiter in the early stages, including the incipient, the mild, the severe, and those extremely severe forms which develop very rapidly and have been described as the acute toxemic type, resembling malignant endocarditis.

2. Typical exophthalmic goiter in cases which may have existed for some time in subacute form with occasional exacerbations, but without marked secondary changes.

TYPES THAT MAY REQUIRE COMBINED TREATMENT.

1. Patients who develop thyroidism after reaching the age of 40 or 50 years:

- a. Those who, after middle life, show thyroidism for a varying period before the appearance of a goiter.

- b. Those who have borne an innocuous goiter for years and late in life develop signs of thyroidism.

2. Atypical forms of thyroidism:

- a. Men and more often women of any age who present many signs of thyroidism, but who often have a dry skin and bradycardia and are usually considered neurotic or neurasthenic.

- b. Patients with nervous and vasomotor signs of thyroidism, but who complain of a more or less constant headache accompanied in most cases by nausea and abdominal discomfort.

- c. The psychopathic cases, the mental disturbance having no definite relation to the severity or type of thyroidism.

The purpose of the classification given above is to serve as an aid in the serum treatment, and in our opinion the groups outlined can not be omitted. Before entering a discussion of the prognosis, the results which we have obtained, and the method of treatment in each separate group, it is necessary to consider briefly some of the simpler fundamentals of the thyroid chemistry.

PHYSIOLOGIC CHEMISTRY OF THYROID PROTEIDS.

There is as yet no agreement among investigators as to the chemistry of the thyroid secretion, and its physiology is even more obscure. Various chemical substances have been isolated from the gland by different methods and it is impossible to reconcile all the statements that are found in the literature. The physiologic activity of the secretion is probably due to the iodine compound which it contains, and we have abundant evidence in the analyses reported by Oswald¹⁷ that the iodine may vary within wide limits, even in health. There has been no demonstration that an iodine-free proteid from the thyroid has any marked physiologic effect. We may believe that the activity of the secretion stands in close relation to its iodine content. It is not known, however, whether the iodine must be in proteid combination to exert its full physiologic action or whether some cleavage product is equally active. The accepted methods of measuring the physiologic value of thyroid preparations do not permit of accurate quantitative distinctions. As a physiologic process the secretion enters the blood or lymph without the intervention of digestion and it seemed to us that its hypodermatic administration would give the best therapeutic results. Saline and glycerin extracts of the gland have, therefore, been preferred and also certain pure proteids have been isolated and kept in solution for hypodermatic administration. The methods followed in making these extracts and proteids were the simplest possible. In the beginning of the work glycerin extracts were preferred, but these caused so marked a local reaction when given hypodermatically that their use was abandoned and a saline extract substituted. This latter solution has been made by thoroughly pulverizing fresh thyroid glands and extracting the hashed mass for from three to five days in five volumes of physiologic salt solution with the addition of a little chloroform to prevent bacterial growth. The extract is filtered first through paper and finally through a Berkefeld candle and sealed in small glass tubes until wanted for administration.

17. Oswald: *Ztschr. f. Physiol. Chem.*, xxxii, 1901, p. 121.

It is not known whether there is more than one iodized proteid in the gland. If one prepares a saline extract of perfectly fresh thyroid glands one finds that a considerable proportion of the proteid therein contained may be precipitated by the addition of acetic acid. From the filtrate a further portion of proteid is precipitated by half saturating with ammonium sulphate. Both these proteids contain iodine and are physiologically active. Oswald¹⁸ maintains that they are identical proteids, and there is certainly much evidence to favor his belief in the case of normal glands. With pathologic glands in the stage of cellular hyperplasia the acetic acid precipitate contains more phosphorus and less iodine than the ammonium sulphate precipitate. With normal glands the amount of the proteid which is precipitated by the acetic acid depends on the freshness of the glands, the concentration of the saline extract made from them and the amount of acid used in the precipitation. Dilute extracts of perfectly normal glands may give no precipitate at all with acetic acid, while a concentrated extract from the same gland yields an abundant precipitate on the addition of acetic acid. Proteids obtained by both methods of precipitation, that is, by acetic acid and by ammonium sulphate, have been administered, but we were dealing with normal glands and it probably made no difference whether we used one or the other, for the evidence indicates that the two precipitates are of the same identical proteid. With pathologic glands, there may be a considerable difference in the therapeutic effect of the two sorts of proteids. The normal thyroid gland differs sharply from other normal glands, such as the liver or kidney, in having a large proteid content which forms no part of a living cell, so that the cell nuclei furnish only a relatively small amount of the proteid in a saline extract. With some of the pathologic glands nuclei are as abundant as they are in liver or kidney and the acetic acid precipitate contains more phosphorus than is obtained from normal gland extract, while the iodine containing globulin precipitated by half saturating with ammonium sulphate is relatively scanty. It is to be expected that proteids having such chemical differences would show differences in therapeutic action. The dose of these pure proteids is very small. We rarely use more than 15 minims of a 1 to 1,000 solution at a single dose, an amount corresponding to the dose of adrenalin. We have been able to get satisfactory therapeutic results with less disturbance of the heart and nervous system by the use of the proteids isolated in this fashion than we have by the use of the commercial

18. Oswald: Die Eiweisskörper der Schilddrüse, *Ztschr. f. Physiol. Chem.*, 1899, xxvii, p. 147.

tablets. The matters relating to the preparation and physiologic value of these proteids are now under investigation and it is quite probable that new methods may be substituted for the ones we have already employed.

These proteids were prepared in the first place in order to alleviate the occasional severe and unpleasant reactions following the use of the antiserum. These reactions occurred more often in those patients who had had the disease for a long time or who had acquired it after middle life. In these two groups atypical cases were occasionally found with some of the symptoms of myxedema, and since the antiserum was not well borne, and because of the many reports in the literature of the successful treatment of such cases by thyroid preparations, we were led to believe that a prothyroid treatment rather than antithyroid treatment was indicated. The hypodermatic administration of thyroid proteids approaches more nearly to physiologic conditions than administration by mouth. Certainly we get more prompt effects by this method and our experience in a large number of cases justifies our belief that pure proteids are more suitable for therapeutic uses than the whole gland substance.

TREATMENT.

In the discussion of the treatment the classification given above will be followed, and the methods and results given under each group. The statistics given in this paper¹⁹ do not include the 105 cases which Dr. Rogers has treated personally, as they are reported in detail at the end of the paper. All of the 141 patients reported herewith, except three which died, have not taken serum for six months and many of them have completed treatment for eighteen months. Serum has been furnished to 126 cases in addition to those which Dr. Rogers has treated, but they are not included in this report for the reasons that some of them are still under treatment and the outcome is undecided, others have not finished treatment for a period of six months, and from a third group satisfactory reports have not been obtained.

Types Favorable for Serum Treatment.—*Group 1.*—Typical exophthalmic goiter in early stages, including the incipient, the mild, the severe, and those extremely severe forms which develop very rapidly and have been described as the acute toxemic type resembling malignant endocarditis.

19. The statistics quoted in the first portion of the article are taken from the records of one of us (S. P. B.) and are from cases which have been for the most part treated under direction by physicians whose private cases they were. The statistics of the cases treated personally by Dr. Rogers are given at the end of the section on treatment.

It is in this group that we have had the largest percentage of complete success, the most prompt, and the most striking results. This is, perhaps, because in these early cases one is dealing with uncomplicated conditions which favor a specific treatment. It is interesting to note that those who favor operative treatment urge the desirability and even necessity of early surgical removal of the gland if the best results are to be obtained. The incipient and mild cases in young people generally yield promptly to serum treatment. It is probable that a large percentage of these two groups would improve by rest and careful hygienic treatment, and the administration of serum should not be a reason for neglecting these factors; but our experience has shown that serum can arrest these cases promptly. In some cases the entire clinical condition has been changed in a week's time and the symptoms of the disease were no longer discoverable. Patients having mild symptoms do not all recover under rest treatment, and the serum has been used in many of these cases with complete success, after the failure of other forms of therapy.

The simple soft goiters which often develop in young women from various causes are at times accompanied by mild symptoms of Graves' disease. Iodin in some form has been for many years the usual drug treatment of these cases, and in a considerable percentage with the happiest results, but some of them, which in the early stages may not be distinguishable from an incipient Graves' disease, are made worse by such treatment and may develop a typical condition of hyperthyroidism. The administration of thyroid to such patients is occasionally of benefit, but whether given as a means of therapy or for the purpose of establishing a diagnosis, thyroid should be given in very small doses and the effect on the patient carefully observed. This matter will be referred to at a later point in the paper. For these two groups, the incipient and the mild, the serum need not be highly active and may be given at intervals of three to five days. In most of these cases a prompt result may be expected.

We have included in this subdivision the very severe, rapidly developing cases which have many of the characters of an acute infection. This type forms only a small percentage of the total number of cases treated, and yet it is in many respects the most interesting. The very marked improvement caused by the administration of a small amount of serum is quite comparable to the effects seen in diphtheria following the administration of antitoxin. In no other group of cases is the toxemic character of the disease so well shown, and it is precisely with these cases that we have had the most convincing clinical evidence of the antitoxic action of the serum.

The recently developed severe cases take the serum with less reaction than the milder cases, and they may in the beginning take more of it. In some instances we have given a small dose every twenty-four hours until four injections have been given and then have increased the interval to two days. It is necessary to watch the patient carefully for unfavorable reactions, and the serum must not be forced in the presence of marked local and general reaction. The rapid development and great severity of these cases is a bar to surgical removal; the failure of general measures is in sharp contrast to the success of the serum treatment and is, in our opinion, one of the best arguments that can be advanced regarding the nature of the disease and the mechanism by which the serum causes improvement.

The majority of the patients in the first group progress very favorably and many of them recover entirely, which means that all symptoms disappear, under doses of about 0.5 c.c. of an active antiserum given at first as often as the reaction permits and then gradually increasing the interval between injections up to five or seven days. In some cases, however, a small dose of antiserum, 3 to 5 minims, not enough to produce any reaction, given once every twenty-four hours for two or three weeks, will sometimes act better. The best of hygienic surroundings are necessary throughout the treatment, and these, it should be said, are not possible in a public hospital ward, with its attending excitement and often distressing circumstances. In some cases a long time is required to effect a cure or even much improvement, and this means an average of two or three months of antiserum treatment and six months more of restful vacation under constant observation.

Exophthalmos and goiter are the last symptoms to disappear, and as long as the latter persists there is some danger of the recrudescence of symptoms under mental or physical strain. The longer a patient remains free from thyroidism, even with a persistent goiter, the less likelihood there seems to be of the recurrence of the disorder. Some kind of infection, generally tonsillitis, is one of the troublesome causes of accidents and exacerbations. While the entire disappearance of the goiter is a result to be earnestly desired, in the majority of cases it can not be obtained quickly by the serum treatment, and as soon as the more distressing symptoms have subsided it is wiser to reduce the serum gradually and depend in part on hygienic and tonic measures. Occasionally, in the latter stages of the recovery, very small doses, $1/50$ or less of a grain, of thyroid proteid act beneficially. If commercial thyroid is used, doses of not more than $1/4$ of a grain should be given and the effect very carefully watched.

In giving the statistics we have divided the cases into three groups, distinguished by cure, improvement and failure. In order to give an intelligent idea of the results it is necessary to define these terms, and this is particularly necessary because reports in the literature show widely varying conceptions of the term "cure" as applied to the treatment of this disease. By a "cured" patient we mean one in whom the subjective symptoms have been entirely relieved and the objective symptoms have subsided to such an extent that nothing more than a gland discoverable only by deep palpation remains. The majority of the improved patients still have a small goiter, but with most of them the subjective symptoms have been relieved, and the patient has been made comfortable and able to work and act in nearly all respects like a normal human being. It is worth noting that with some of the improved cases the improvement continues slowly, although the treatment may have stopped for a year or more. The failures are those cases in which the treatment has produced no beneficial effect.

Of the total of 141 cases reported, 52 belong to the first group of the classification. Of these, 18 have been cured, 28 improved (all but 2 being very much improved) and 6 have failed to improve. Of the 6 failures, 3 cases resulted fatally. It should be stated here also that 6 patients properly belonging in this group have applied for serum, which was forwarded as promptly as possible, but in the interval the case terminated fatally before the serum could be given. Such sudden terminations are likely to happen, especially in the very acute form of the disease. Of the three fatal cases listed with the failures, two received only two injections of serum and the third received only one injection. They were of the same acute type as the six cases which terminated fatally before the serum was received. It is impossible to include in a paper of this sort a case history for each patient treated, but the details of a typical cured, an improved and a fatal case are appended herewith.

CASE 1: CURE.—Patient.—S. H. C., woman, 39 years old, always well except for attack of grip nine weeks before the development of thyroid symptoms.

Signs and Symptoms.—This was a typical acute case, with marked tachycardia; the patient was very nervous, had marked tremor and diarrhea, perspired freely, had troublesome thirst and did not sleep well. Her skin was markedly pigmented; she had marked exophthalmos and a small soft goiter gradually increasing in size. The symptoms of the disease were on the increase; the patient has emaciated somewhat but she is able to be up.

Treatment.—The patient was seen by two prominent clinicians who pronounced it a typical acute case and advised serum treatment. Previous to these consultations she had been treated with rest and tincture of belladonna without beneficial effect.

Result.—The patient was under serum treatment from March 20 to August 31 and continued to improve steadily. The final report received in December states that the very best results were obtained. The gland was normal in size,

tachycardia entirely absent, the exophthalmos cured and the patient stouter than she ever was before and feeling perfectly well. Her color was good, and the appetite and digestion as good as they ever were in her life.

CASE 2: IMPROVEMENT.—*Patient.*—R. M. V., woman, aged 36, married, weight 163, had pneumonia at 17, otherwise healthy.

Signs and Symptoms.—Symptoms first noticed about a year before the treatment began and the patient had lost 94 pounds in weight during this time. The pulse was 108 when the patient was in bed and quiet, of fair quality and regular. The heart was dilated, the apex-beat being one and one-half inches to the left of the mid-clavicular line, faint systolic blow at the apex. The patient was very nervous with marked tremor; perspired freely. She had fair appetite with no vomiting or diarrhea at the time treatment was begun. She was always thirsty. The circumference of the neck at greatest point was 15 inches; a soft goiter of moderate size.

Treatment.—Before serum treatment the patient had been receiving iron, tonics, digitalis and Merck's antithyroidin, but the condition had been stationary for some months. The patient was confined to bed. Treatment was begun in April and continued until September.

Result.—The patient recovered in nearly every particular. There was no noticeable exophthalmos; the goiter could scarcely be seen, but could be felt by careful palpation. The nervousness and tremor were entirely gone. The patient now did her own work and her pulse ran from 84 to 90 after a day's work. There was no systolic murmur and the heart was not dilated. The patient feels perfectly well.

CASE 3: FAILURE.—*Patient.*—I. W. K., woman, 27 years old.

Signs and Symptoms.—This was a typical case with rather slow onset, pulse ranging from 180 to 200, heart symptoms very marked, precordial pain, loud systolic blow, heart dilated, apex beat displaced to the left two and one-half inches. The patient had a hacking cough; her voice was somewhat husky, moderately nervous, with a fine tremor. She slept badly, perspired profusely and had a troublesome thirst. The exophthalmos was moderate; the gland varied in size, at times being small and hard and at others large and soft. There was extreme emaciation, the patient had been in bed three weeks and was very ill when the injections were begun.

Treatment.—This was begun on December 20. Three injections were given by January 4, on which date the patient died.

Result.—There did not seem to be any reaction in either way to the serum.

CASE 4: MARKED IMPROVEMENT WITH SUDDEN DEATH LATER.—This case is very interesting and is included because the manner of death is typical of some of the acute cases.

Patient.—P. J. D., young woman, aged 19, single, history of Graves' disease extending over two and one-half years. The onset of the disease, occasioned by a severe fright, was rapid and progressive.

Signs and Symptoms.—The pulse was 148 and fairly regular; the chest bulged at each pulsation; there was some precordial pain; no heart murmurs; the heart not dilated. Respirations were 27 per minute; there was a slight cough; the voice quite clear. The patient had until recently perspired profusely; her appetite was fairly good and there was no diarrhea. Menstruation had been interrupted for nine months. The patient had become reduced from 145 pounds to 75 pounds in weight. She sat up, but the condition had remained stationary for some weeks. The patient was very nervous with marked tremor.

Treatment.—Up to a month previous to the beginning of the serum treatment the patient had been given thyroid extract. Treatment by serum was given from

November until the following June. The patient began to improve immediately after beginning serum and continued to an almost complete recovery.

Result.—The following report of the patient's condition on September 13 was made: The patient weighs 144 pounds, is able to walk several miles without fatigue, has been doing all the housework for several weeks past. Exophthalmos is noticeable but not marked. Heart is regular, 94, no murmur. The patient is not nervous and has no tremor; sleeps well; bowels and menstruation regular.

Outcome of Case.—The patient died suddenly on October 28 after having had a severe pain in the head for a few hours. There was no autopsy.

Types Favorable for Serum Treatment—Group 2.—Typical exophthalmic goiter in cases which have existed for some time in subacute form with occasional exacerbations but without marked secondary changes.

These cases are, as a rule, amenable to serum treatment, but there is generally not such rapid improvement, and the injections must be continued over a longer period than with the acute forms. In some instances in which the serum has been begun during the period of exacerbation there has been almost immediate relief, in this respect resembling the acute conditions, but permanent results were obtained only after some months. The longer the duration of these cases the slower the patients are to show permanent improvement, but, since we have had complete success with some cases of ten to twelve years' duration, we are led to the conclusion that it is the point to which the disease has progressed rather than the number of years of its course which is the determining factor. If treatment is begun during an exacerbation it should be carried out in much the same manner as with the preceding group, that is, the early acute cases, but it will probably be necessary to continue administration of a weak serum at intervals of five days to two weeks for some months before normal, stable equilibrium is attained.

Of the total of 141 cases reported, 70 have been in this group. Of this number, 16 have been cured, 36 have been greatly improved, and there have been 18 failures, 5 of the latter being fatal. Six patients properly belonging in this group have had a preliminary course of serum treatment and have later been operated on. Three persisted in the serum treatment for a sufficient time to give it a fair trial, with the result that one of them was much benefited and later was entirely cured by operation, while with the two others no decided benefit was obtained and operation, undertaken later, was fatal. The 3 remaining in this group of 6 did not persist long enough in the use of serum to permit any conclusion as to its value, and the subsequent operation proved fatal in each case. The three cases first mentioned as having had serum for some time are included in the list of 141 cases, one as improved and two

of them as failures. The outcome of the operation in the 5 fatal cases can not be ascribed to the preliminary use of serum. Case histories of typical cases occurring in this group are given below.

CASE 5: CURE.—*Patient.*—Mrs. B., aged 44, marked case with history of six years of chronic thyroidism.

Signs and Symptoms.—Pulse 148, irregular; heart dilated, apex $5\frac{1}{2}$ inches from midsternal line; patient very nervous, marked tremor; slept very badly; troublesome thirst; skin pigmented; moderate exophthalmos; irregular menses. Patient emaciated; weight 106 pounds; gland small but firm. Patient confined to bed.

Result of Treatment.—Treatment was given from February to September. In November her physician reported that he considered the patient cured. Her pulse averaged 74; the thyroid could be felt on deep palpation; the patient was not nervous and had no tremors. Exophthalmos could not be detected. The patient weighed 132 pounds, felt in fine spirits, was doing her own work and as far as could be detected was perfectly well.

CASE 6: CURE.—*Patient.*—Mrs. H., 54 years old, member of a highly nervous family.

Signs and Symptoms.—The patient was very excitable and nervous, melancholic at times; had attacks of cardiac dilatation and it was necessary to use morphin to relieve pain about the heart. There was very marked exophthalmos, progressive enlargement of the thyroid, very marked tremor, temperature from 99 to 100, great prostration, and pulse 110 to 140 per minute. The patient's condition was grave for several months. She was under medical treatment for three months and at times showed some symptomatic improvement, but nothing permanent.

Result.—The patient was under treatment for six months. At first she made slow but certain improvement, which has continued progressively, and at the time of the report, two years after beginning the treatment, the patient was well. There was no exophthalmos and no tremor; the patient was not nervous; the pulse was 84, regular, and of good quality; the thyroid could barely be made out, and there had been a great gain in weight, the patient now weighing 175 pounds. The physician considered her completely cured.

CASE 7: IMPROVEMENT.—*Patient.*—Mrs. C. F., aged 46.

Signs and Symptoms.—The patient was extremely nervous; had very marked tremor; slept wretchedly; had troublesome thirst and diarrhea at times. The pulse was 120 to 160; there was pronounced exophthalmos; the gland was only slightly enlarged. The patient had lost 59 pounds, was just able to be up a short time during the day and had lost control of the rectum. She had been very nervous for 12 years, and the physician spoke of her as hopeless.

Result.—Treatment was given from May until October. A great improvement was effected. The pulse was from 80 to 90; the tremor was almost completely gone. The patient made a gain of 30 pounds in weight during this time, was not nervous and slept well. The rectum was under perfect control. The patient was doing light housework and complained of nothing but occasional palpitation on climbing the stairs, but there were weeks when this was not noticed. There was distress at times from flatus in the stomach. Exophthalmos was still noticeable, but not nearly so marked. The size of the gland was about the same. A later report states that the improvement has continued.

CASE 8: IMPROVEMENT.—*Patient.*—Mrs. I., aged 38, had goiter for twelve years, gradually increasing in size. During the last three years the symptoms had gradually been growing worse.

Signs and Symptoms.—The goiter was large, firm, low down on the right side; there was little exophthalmos, marked tremor, pulse when quiet 110, no heart murmur and marked dyspnea on exertion. The patient was very nervous, perspired very freely, did not sleep well and had a variable appetite with occasional diarrhea.

Results.—The patient was under treatment from February to August. There was not much diminution in the size of the gland. The dyspnea was entirely relieved so that the patient was able to do her own work and go out walking for a long distance, which she had been unable to do for years. The heart came down to 80, nervousness and perspiration were entirely relieved, and the patient improved so much that she discontinued treatment.

CASE 9: FAILURE.—*Patient.*—Mrs. M., aged 54; first symptom noticed in 1896. The patient suffered from nervous strain and worry immediately preceding the acute attack.

Signs and Symptoms.—The pulse was 108; there was precordial pain and systolic murmur over the base of the heart. The patient was not nervous and had no tremor; slept fairly well and had a fair appetite. She had a small soft goiter and slight exophthalmos; had lost considerable weight. The most troublesome symptoms were vomiting and diarrhea, also edema of the face and hands. (Diagnosis was questionable.)

Results.—The patient was under treatment from May to October. The serum relieved the gastrointestinal symptoms very decidedly, and the patient was for a time very much improved.

Outcome of Case.—The heart did not improve much, however, and later it steadily grew worse and the patient died in a fatal attack of syncope.

Types That May Require Combined Treatment.—By the combined treatment we mean the administration of both the antiserum and a pure thyroid proteid. The administration of these substances need not be simultaneous, since at one period, generally the beginning of the treatment, the use of antiserum gives the best results, and at a later period the additional administration of a small amount of thyroid proteid is necessary to continue the improvement. If the case presents fairly typical symptoms of Graves' disease, we begin by giving small doses of serum, and in many cases find this treatment followed by very satisfactory relief, but if the serum causes very severe or disagreeable reactions and if there is no corresponding improvement in symptoms we begin the addition of small amounts of thyroid proteid.

In a considerable number of these cases it has been our experience that the administration daily of a small amount of thyroid proteid, three doses of 1/50 of a grain, and the injection of a small amount of serum, 5 minims, every fifth day gives the best results. It is not easy to understand precisely why this is true, since it is paradoxical to believe that a hypothyroid condition exists in the same patient that has symptoms of hyperthyroidism. It may be that the thyroid proteid from a normal healthy animal answers the physiologic need of the patient for such a secretion, while the abnormal product from the patient's own

gland is neutralized by the use of serum. It is certain that the serum does not neutralize the physiologic action of a sheep thyroid proteid; so that in effect we may, by giving serum and the proteid from normal sheep thyroid simultaneously, substitute the action of the normal secretion from the animal for that of the abnormal secretion from the patient's gland. Whatever may be the explanation, the clinical fact remains that some of these mixed cases improve more rapidly and surely by such a method of mixed treatment.

It is impossible to formulate precise rules to be followed in administering serum to this group of patients, for the reason that the type and clinical conditions are so variable, but from a study of the case we may be able to decide whether the symptoms are a manifestation of hypothyroidism or hyperthyroidism, and the beginning of the treatment should be made in accordance with these conclusions. In my opinion, the logical treatment for the group of mixed cases is the combined treatment, as it combats the dysthyroidism, which is probably an essential factor in the production of the symptom-complex. The treatment of the atypical and the advanced typical cases requires a finer discrimination and more accurate judgment in the employment of antiserum and thyroid preparations than is needed in the treatment of typical cases which form our first group, and it must be admitted that some degree of experimentation must be made with some of these patients before the most suitable treatment is determined on. Complete recovery is slow, but encouraging improvement is often noted in a comparatively short time.

Before we can give treatment intelligently in cases belonging to this group a very careful study must be made of the past history of the disease, the symptoms and the clinical condition in order to determine, if possible, whether the hypothyroid or the hyperthyroid effects predominate, for our therapeutic efforts will be regulated accordingly. The manifestations of these two conditions are so complex and our means of diagnosis so incomplete that we can not always decide in which group a given patient belongs. In most instances it is possible to classify the case, but occasionally we find one of a mixed type that is very difficult to explain.

It will be noted that we have included under the group that may need combined treatment the atypical cases and cases of those patients who develop thyroidism comparatively late in life. The patients in atypical cases are not necessarily advanced in years, many of them being under 30, and the typical patients over 50 do not all need combined treatment, so that the factor of age can not be the criterion by which the matter is decided.

The cases belonging in the atypical group are much the most difficult to classify and treat. None of them have all the typical symptoms of Graves' disease, and the majority of them appear to suffer from an abnormal secretion rather than a pure hyperthyroidism. They have often come to us with the diagnosis of Graves' disease because they have at one time had a typical form of the disease, but as we see them they have passed out of that typical form to the mixed type and occasionally one appears to have a nearly complete myxedema. Such cases are not to be treated by serum alone. Surgery is equally out of the question or should be with most of them.

In order to bring the matter more directly in view we append a table of contrasts to show the differing symptoms of hypothyroidism and hyperthyroidism. For the hyperthyroidism typical Graves' disease is the best example, but for the opposite type we have selected the myxedematoid rather than typical myxedema in advanced stages.

HYPERTHYROIDISM.	HYPOTHYROIDISM.
AGE.	AGE.
More common in young women, 18-30.	More common in advancing years, 35-50.
ONSET.	ONSET.
May be slow and gradual or sudden and acute.	Slow and gradual, in many cases engrafted on an old Graves' disease.
HEART.	HEART.
Tachycardia 120-180; pounding beat felt over wide area; often a loud systolic murmur over apex, base and along the great vessels. Irregular and very susceptible to the effects of exercise. Blood pressure variable, generally low, pulse soft and full, marked dyspnea on slight exertion. Marked edema of legs.	Rarely above 100, may be irregular with heaving impulse. Pulse generally shows high tension and the blood pressure is above normal.
NERVOUS SYSTEM.	NERVOUS SYSTEM.
Fine tremor affecting nearly all the muscles, twitching, and occasionally spasms. Patients are abnormally irritable and excitable, apprehensive, mentally very active and physically restless. Muscular weakness prominent.	Patient may have some tremor, and muscular weakness is likely to be very pronounced, but there is not the same restlessness and jactitation. Patients are occasionally irritable, but they are generally rather dull and apathetic, mentally slow; memory defective. Pains in joints frequent, and there is a marked tendency toward sudden giving-way of the legs when walking.
EYE SIGNS.	EYE SIGNS.
Exophthalmos generally present, although it is not invariable. Occasionally unilateral, corresponding to the side having the enlarged thyroid lobe. Various symptoms arise in consequence of the exophthalmos. No pupillary changes.	Exophthalmos is unusual, although it may have been present at one time.

GLAND.

Enlargement varies from nothing to very large goiter. The blood vessels over the gland are generally much enlarged and pulsate markedly. Right lobe generally the larger.

Often no enlargement can be made out; when there is a goiter it has an elastic, rubber-like consistence, occasionally cystic and nodular, but very distinctly different from the active, pulsating gland of Graves' disease.

NUTRITION.

Severely disturbed; in most cases there is a loss in weight, which may progress to extreme emaciation. Appetite variable; vomiting and diarrhea frequent complications. Patients drink a great deal of water.

Generally not seriously disturbed; patients hold their weight and in most cases gain slowly; constipation rather than diarrhea, and flatulency a troublesome habit. Patients do not drink much water.

SKIN.

Profuse perspiration, erythema, urticaria, dermatographia; pigmentation, which may occasionally be so marked as to suggest Addison's disease. Hair falls out, but is not coarse and dry. Patients prefer thin clothing and cold rooms. They are more comfortable during cold weather than during hot weather.

Dry, may be scaly; patients do not perspire on exertion; hair dry, brittle, scalp scaly. Pigmentation not common. Patients prefer thick, warm clothing and are cold most of the time. Much more comfortable during hot weather.

TEMPERATURE.

May be only slightly elevated, 99-100. With severe acute cases it runs often to 102-104.

Subnormal, may reach as low as 95.

URINARY FINDINGS.

In most cases normal in volume; glycosuria not unusual; polyuria often observed in later stages. Nitrogen partitions show a very much decreased kreatinin excretion, while kreatin is present in large amounts. Nitrogen loss is marked during the period of emaciation.

Albuminuria not unusual. Nitrogen partitions do not show so marked a disturbance in kreatinin and kreatin ratios. In large number of cases urine practically normal.

BLOOD.

Hemoglobin low, leucopenia in severe cases, with a marked relative lymphocytosis.

Hemoglobin low, white blood count normal.

MENSES.

Very irregular or completely suppressed.

Generally regular but scanty.

The atypical cases do not fall sharply into one or the other of these groups, but show most unusual combinations of symptoms of pure Graves' disease with those of myxedema. The existence of such cases has been recognized for some years and their successful treatment with thyroid preparations is not new. It has been our observation, however, that a large percentage of these patients do not do well on thyroid extract alone, and that a combination of serum with thyroid proteid gives the best clinical results.

The statistics which are given under the last two groups do not furnish an accurate estimate of the prognosis in those types because we have used the combined treatment only during the last year and there are comparatively few who have finished the treatment for a period of six months. My list shows 19 cases which fill this requirement, however, and of these 10 have been greatly improved and 9 have failed of improvement. There have been no deaths in this group and no patients have been cured. It is only fair to state that there have been 4 cured patients, but they have not finished treatment for a period of six months as yet and so they have not been included.

CASE 10: IMPROVEMENT.—*Patient.*—Miss H., aged 40, had had subacute symptoms for ten years.

Signs and Symptoms.—The pulse was 120 to 140; respirations about 20 and labored; voice rather husky. The patient was rather nervous; had no tremor; could sleep only in a semirecumbent position; had a good appetite; had diarrhea, pronounced exophthalmos, a small soft goiter, and was slightly emaciated. Her condition was stationary for some time.

Results of Treatment.—The serum was used for six weeks with some general improvement in the condition of the heart, but the gastrointestinal condition was not relieved. During the next six weeks the combined treatment was given with the best results. The pulse dropped to 98, exophthalmos was much less, the diarrhea completely relieved, the thyroid much softer and almost normal in size. The patient slept well in recumbent position; was rapidly improving and felt equal to resuming her occupation as a teacher. A later report states that her satisfactory improvement has continued.

CASE 11: IMPROVEMENT.—*Patient.*—Miss N., aged 38; history of Graves' disease for six years.

Signs and Symptoms.—The pulse was 120 to 130; there was slight precordial pain and tremor, especially pronounced in the hands and knees, and the appetite was poor. The patient was very nervous, slept badly and was troubled with diarrhea and sometimes with vomiting; had marked exophthalmos, a small hard goiter; was emaciated and not able to be up.

Results of Treatment.—Treatment was continued from May until October with the result that the patient gained 30 pounds in weight; pulse became regular at from 80 to 90; the appetite good; no diarrhea; menses regular; nervousness entirely relieved. "In short, her general condition is very good."

CASE 12: FAILURE.—*Patient.*—Mrs. G., aged 56; history of goiter for twenty years with symptoms of an atypical Graves' disease for eight years.

Signs and Symptoms.—Patient was nervous; did not sleep well and had a poor appetite with occasional diarrhea. Pulse was from 90 to 100, regular and of fairly good quality. There was no troublesome perspiration or unusual thirst. The patient had had moderate exophthalmos in the past, but this symptom was no longer seen. Headache was a troublesome feature of the case. Temperature was normal.

Results of Treatment.—The case was not considered a favorable one for treatment with serum, but it was begun cautiously and was carried out for some time with no benefit. Later the combined treatment was tried for a period of six weeks without benefit. Patient was no better in any way than at the beginning of the treatment.

The patients personally treated by Dr. Rogers to Jan. 1, 1908, number 105, who presented, more or less markedly, the three cardinal signs of exophthalmos, goiter and tachycardia. Reports could be submitted on about 100 additional cases, but, as they were mostly hospital or consultation patients whose records are incomplete or people who presented irregular and doubtful forms of thyroidism, it has seemed best to confine the report to those that have been personally treated throughout and to typical examples of exophthalmic goiter. There is thus no question about the diagnosis and it gives a clearer understanding of what has been accomplished for these sufferers.

Of the 105 patients, 12 have been cured of every trace of disease, including both exophthalmos and goiter, 17 have no symptoms of a pathologic nature whatever except a small goiter in a few instances, and one has a slight right-sided exophthalmos but no goiter. As neither of these abnormalities is noticeable without close examination, I (J. R.) think it is fair to group these cases together and state that a total of 30 have been cured.

Forty-three who have undergone specific serum or combined treatment have been improved, and, while some of these are still under treatment, many of them are expected to be ultimately in the cured class.

Four have failed to show any benefit by specific treatment.

Seven whose condition in several instances was at first greatly benefited by the antiserum have died from the natural progress of the disease.

Twenty-one have been operated on either before or after undergoing the specific treatment described below, with the result that none can be called a perfect cure, and two of these 21 have died as a direct result of the operation.

A brief analysis of the failures, the deaths and the operative cases presents much that is instructive. The four described as failures under antiserum received no other treatment and occurred early in our experience before we had grasped the importance of supplementary thyroid treatment at certain stages, or in certain forms of the disease. Nevertheless, in so complicated and obscure a disease it is only reasonable to expect a considerable percentage of failures for a long time to come, or until there is a much more definite understanding of the physiology of the thyroid than exists at present.

One of the most striking features in a survey of all the 105 cases is the gravity of the prognosis, for, in spite of everything which could be done, there was a death rate in this comparatively brief period of observation of less than three years, of approximately 10 per cent. One

gains the impression from the observation of many patients in different stages of exophthalmic goiter that the mortality under ordinary medical treatment is considerably higher than this. Indeed, the expectation of life after the onset of the symptoms in a well-marked case can not be more than ten or twelve years.

The best results occurred in the very severe acute or the early mild cases, especially if the goiter was small. The failures and deaths occurred in cases of marked severity with large goiters and after the disease had been manifest for several years or in patients who developed it late in life. There have, however, been some exceptions to these rules, as a few with small goiters have done poorly while others which appeared almost hopeless have progressed surprisingly well. Five women while in the improved class have gone through a normal pregnancy with continued gain, and all but one were delivered at full term of healthy children. Antiserum was given in three cases throughout the pregnancy; the other two were so well during this period that no treatment was required.

Two of the five mothers can now be placed in the cured group, though one still has a barely perceptible goiter, but no other symptoms. The surviving children show no abnormality. In reviewing the histories of all the patients, however, the heredity was quite apparent in a considerable number of the cases. It was not unusual to elicit a more or less close family history of goiter and in a few cases of true exophthalmic goiter, but the proportion was not greater than in other diseases like tuberculosis, rheumatism or cancer.

Two patients presenting a well-marked though not particularly severe type of the disorder of several years' duration made an almost complete recovery after a few months of antiserum treatment. The exophthalmos, tachycardia and subjective symptoms entirely disappeared, but the goiter remained quite large, though softer than at the time of the first observation. During this period a considerable degree of rest, quiet and good hygiene had been enforced, but as soon as the patients felt well work was resumed, by one as a book canvasser, by the other as a tailor in a sweat-shop, and very soon thereafter during a time of hot weather and hard work, a combination which seems particularly trying, both patients relapsed. Antiserum seemed to do harm, prothyroid treatment was without avail, and the disease progressed gradually to a fatal termination which was preceded by stupor and coma.

A third patient had almost the same history, but by a careful use of antiserum and combined treatment, apparently with beneficial results, barely escaped death and has now no symptoms except of small goiter.

Of the 21 patients operated on 3 had only the superior thyroid vessels

ligated. The 18 remaining patients underwent the more radical and generally recommended operation of removal of a portion of the thyroid gland either before or after submitting to the specific thyroid medication. And these 18 are the most important from a surgical standpoint.

Nine patients had been operated on and had relapsed before coming under observation, and some of them had reached a condition worse than that which had called for the interference. Three of these nine had each undergone two operations with no lasting benefit. Of the nine who relapsed after radical operation, four have been greatly benefited by the antiserum or by prothyroid treatment and might be called cured if sufficient time had elapsed to demonstrate that another relapse will not occur. Three have so much improved as not to be conscious of illness, although some tachycardia is still perceptible. One (who has had two operations) completely failed to improve in spite of careful treatment and is slowly but surely losing ground, and the other, who also had had two operations, failed entirely under the antiserum, and in July, 1907, suffered a removal of the second thyroid lobe, leaving only the isthmus. This, in November, 1907, was evidently enlarging, and, though the patient had after the last operation, as after the previous two, gained considerably in flesh and strength, both subjective and objective symptoms were very apparent, and she stated that she felt little, if any, better.

The remaining nine patients, after failing to improve under the antiserum treatment, suffered removal of one lobe and the isthmus of the thyroid gland either at the hands of Dr. Rogers or at those of other surgeons. Of these, two died of acute thyroidism (?) shortly after the operation. The first came under observation early in my experience with a very severe toxemic form of the disease, and when the antiserum was begun death seemed imminent. After two or three months of antiserum treatment she almost entirely recovered, but still had comparatively mild symptoms and a pulse rate of 110. In an attempt to complete the cure, the right lobe and isthmus were removed with a fatal result. The other death occurred in a psychopathic case with mild symptoms of Graves' disease and melancholic depression, with delusions of sight and hearing. Antithyroid and prothyroid treatment were both tried and seemed only to make the psychosis worse, so removal of one lobe of the much hypertrophied gland was attempted and a fatality followed within two days.

The seven remaining patients were all treated with antiserum with more rather than less unfavorable results, although five were at first greatly improved and then remained stationary or were made worse by the medication. I believe now, however, that with time and good hygiene and the judicious exhibition of the combined treatment, the advantages of

which have only of late become apparent, much more could have been accomplished. Nevertheless seven patients, after failing to recover under antiserum treatment, had one lobe and the isthmus of the gland successfully removed, and the last operation on these cases was in October, 1907. Only three of the seven were much benefited by the operation. One who was operated on in November, 1906, remained comparatively well though unable to undergo vigorous exertion until February, 1908. The second patient, after gaining somewhat under antiserum, relapsed and had the left superior thyroid vessels tied and the right lobe removed in Stockholm in September, 1906. After this she gained slowly in flesh and strength and returned to this country, but suffered another relapse with typical symptoms in November, 1907. The third patient came under observation in July, 1907, and refused operation after appreciable benefit from the antiserum, especially in the very troublesome insomnia and weakness. He then left the hospital and relapsed in December, and in January, 1908, had one lobe and the isthmus removed, and, although much improved, still shows in April the characteristic symptoms.

The other four of the seven patients who underwent operation after failure with the antiserum are about as they were when first seen and at least two of them were for several days after the operation in a rather critical condition and convalesced to their original state very slowly.

These experiences, while not very extensive, have seemed to indicate that when antithyroid, or in very exceptional cases, prothyroid treatment fails, not much can be promised by radical surgery. Not only the danger but the uncertainty of the result of operative removal of part of the thyroid in exophthalmic goiter demands that until there is a more definite knowledge of the physiology and pathology of the organ, at least a trial should be made of the specific treatment.

According to these statistics about 30 per cent. of all cases under careful management can be practically cured and the earlier they come under treatment the better the prognosis. About 50 per cent. can be improved; some 20 per cent. have failed and of these 10 per cent. have already died. This includes all stages and varieties of the disease; the youngest patient was 5 years of age and the oldest over 80; many have been sent for specific treatment as a last resort and have been considered hopeless at the outset, but the inclusion of all patients without distinction so long as the diagnosis is unmistakable, is the only method by which a reasonable idea of the prognosis can be derived.

THE REACTION FOLLOWING THE INJECTION OF SERUM.

The serum is always given by hypodermatic injection, and we have chosen the arm as the site of injection because it is more convenient for

the patient and because the local reaction causes less trouble in this region and may be treated more readily. The upper arm just below the deltoid should be carefully cleaned and the injection made subcutaneously but not intramuscularly in order to avoid too rapid absorption. In 95 per cent. of the injections the local reaction consists only of an area of hyperemia and slight induration which may be somewhat tender on pressure for a few hours. It quickly clears up and in thirty-six to forty-eight hours the arm is perfectly normal. The indurated area may in some instances be three or four inches in diameter and occasionally the whole arm has become edematous from the shoulder to the finger-tips. Such a reaction is unpleasant but fortunately it is a rare complication and if the arm is wrapped in a wet dressing the reaction subsides without unpleasant after-effects. The exact nature of the reaction in any given case cannot be foretold because the matter of personal idiosyncrasy of the patient is an exceedingly important factor. It is best, therefore, to start with a small dose and to determine the nature of the reaction in each case before the full therapeutic dose is attempted. As has already been stated, the very acute toxic cases take the serum better than the mild cases and with them it may be best to begin with a full dose. If the local reaction is marked it is best to keep hot applications on the arm for half to three-quarters of an hour after the injection, and gently massage the area about the point of puncture. Unless some quite unusual condition results no further treatment is necessary, for the condition subsides promptly. If a second injection is made before the reaction from the first has subsided a more decided reaction is produced in the second instance and the area of the first injection is again excited. The local reaction is therefore of value as a guide in the determination of dose and frequency of administration. The two arms should be used alternately at the site of injection.

The general reaction likewise shows considerable variation. In a large percentage of cases there is no disturbance whatever; there may be, however, a slight rise in temperature accompanied by nausea, some restlessness and perhaps some increase in the tachycardia. Rarely the patient may vomit and all the symptoms of the disease be temporarily exaggerated. If the serum is given too frequently or in too large doses both the local and the general reactions become more severe. The serum must never be pushed in the presence of a progressively increasing reaction. Serious consequences may arise if this precaution is not observed. If, during the course of treatment, an usually severe reaction has been obtained it is best to allow a somewhat longer interval before the next injection, and at the same time to reduce the dose.

During the last two years the study of the phenomena of anaphylaxis¹⁹ and of the so-called "serum disease" has directed attention to the complications of serum administration. Several instances of severe reaction, some of them even with fatal outcome,²⁰ have been published recently and it is evident that serious consequences may occasionally arise. (None of the reactions to which reference is made have occurred in the use of the thyroid serum.) We have tried to avoid such possibilities by giving the serum in the first portion of the treatment at short intervals, that is, from one to five days, and thus avoid sensitizing the patient. In spite of these precautions we have had out of the several thousand injections that have been made in the course of the last three years, a few very severe, and, for the time, alarming reactions, although none have been fatal. These have been obtained in cases which have had the serum for some time and have occurred without any previous warning. (It is only fair to state that nearly all of the reactions occurred in the earlier part of our experience when we were using somewhat larger doses than we now find necessary.) These reactions have developed in most cases very shortly after the injection of the serum, but in a few instances the onset has been delayed for a couple of hours. They have always occurred in patients who have had some previous treatment with the serum. The reaction develops rapidly and resembles in some respects the condition recently described by Ohlmacher²¹ as occurring in two cases treated with antistreptococcic serum. The cutaneous surfaces of the face became very much swollen, the nose, lips and ears were of twice the normal size and the exophthalmos was likewise much exaggerated. There has been some subjective distress, particularly in breathing and the patient has become markedly cyanosed with a rapid, feeble pulse and a more or less pronounced syncope. The reaction is alarming for both patient and physician; but in our experience the condition has rapidly improved and an hour later all evidences of the attack may have passed off. We do not know why such a reaction should develop but it is probably related to the phenomena of anaphylaxis. There is probably some peculiarly susceptible condition of the patient at the particular time the injection is given, for the patient may

19. Rosenau and Anderson: A New Toxic Action of Horse Serum, *Jour. Med. Research*, 1906, xv.

20. Gillette, H. F.: Diphtheria Antitoxin in Bronchial Asthma, *Jour. Am. Med. Assn.*, 1908. Wiley, S. N.: Sudden Death from Injection of Diphtheria Antitoxin, *Jour. Am. Med. Assn.*, 1908, 1, 137. Boone, E. L.: Sudden Death Following Use of Diphtheria Antitoxin, *Jour. Am. Med. Assn.*, 1908, 1, 44. Quigley, I. K.: Collapse After Use of Diphtheria Antitoxin, *Jour. Am. Med. Assn.*, 1908, 1, 768.

21. Ohlmacher, A. P.: Two Instances of Severe Non-fatal Serum Reaction, *Jour. Am. Med. Assn.*, 1908, 1, 875.

have taken the same serum previously without any marked reaction, and may continue to take it again for weeks without ever showing again a reaction at all like this. We have learned to combat these severe reactions with the hypodermatic injection of thyroid proteid, but their great infrequency makes it probable that very few physicians will ever have occasion to deal with them. Since we have given the serum in smaller doses at shorter intervals we have seen few disturbances other than the local reaction in the arm. Following such a reaction no serum should be given for a week and then a somewhat smaller dose should be given for the first injection. The serum produced in each animal varies somewhat from that of other animals so that it is necessary to know the character of the serum and the condition of the patient before the question of dosage can be decided.

The relation which the specific treatment bears to the surgical treatment is naturally of much interest. The list of 141 patients includes 8 who have had some surgical procedure for the condition. Six of these cases have already been described. The two others are interesting as they both are instances of recurrence after operation; one of them is of especial interest as it is the first case in this country of a patient undergoing surgical treatment for the relief of Graves' disease. Brief histories of these two cases are given below:

CASE 1.—Patient.—Mr. F., aged 28, had the disease for eight years, operated on; had the right lobe, isthmus and part of the left lobe removed, "improving the fast pulse, exophthalmos and nervousness." Still had attacks of diarrhea and tremor; pulse was 109; the patient slept badly; had lost 35 pounds in the last three months and was unable to work.

Results of Treatment.—The patient was under treatment for six weeks. Improvement began with the first injection; the tumor was greatly reduced, diarrhea cured, tremor greatly reduced, he can walk a good distance without undue fatigue. Has gained 27 pounds since the treatment began and is in every way much improved.

CASE 2.—Patient.—A woman of 34 years, weight 130, physical condition typical of Graves' disease, tachycardia of 116 to 140, heart otherwise normal, nervous with marked tremor, small goiter of medium consistence, increasing in size during the last few weeks.

History.—This patient was the first to have removal of gland in this country for the relief of Graves' disease. The operation was done by Dr. A. C. Bernays in St. Louis May 13, 1894. The right half of the gland was removed and the superior artery tied on the left side. The condition previous to the operation was serious, but the patient was entirely relieved and remained well during thirteen years following the operation. There was redevelopment of the disease in the spring of 1907. Her condition has been growing worse. Treatment by rest, ice pack and quinin hydrobromate had not improved the condition.

Results of Treatment.—Ten injections of serum were given; the first caused a marked reaction, the second much less, and it gradually decreased to no reaction whatever, "and with this the pulse kept growing slower, she gained in weight, the goiter grew less, and every symptom gradually disappeared."

To summarize these cases again, it will be recalled that five patients tried serum first without benefit and later died as a result of operation; two were operated on before the serum treatment with good result and were later treated successfully with serum for a recurrence of the disease, and the last was benefited considerably by serum treatment preliminary to a completely successful operation. As far as these figures go it would seem that if a case can not be benefited by serum it may be dangerous to operate; and also that, if an operation is likely to be successful serum may also be successful. It appears to be true that the type of case which can be completely cured by operation is a type favorable for serum treatment.

It must not be understood that what has been said above is a criticism of or an argument against the operative treatment. The few brilliant operators who have had sufficient experience to select the cases intelligently, give the proper preliminary treatment, and carry out the operative procedure with the requisite skill and judgment have had very good results and their statistics speak for themselves; but the fact remains that for the majority of surgeons the operation gives a high mortality and not fully satisfactory postoperative results. Moreover, I think the time has not yet come to determine precisely what the results of operative procedure are to be. Surgical removal of the thyroid for Graves' disease can scarcely be considered an emergency operation, and in my opinion should not be undertaken unless the operator is thoroughly familiar with disease, and has had his judgment tempered by a wide experience.

Graves' disease is a serious malady and should be so regarded. The fact that incipient cases in young women will in most cases improve promptly with rest and tonic treatment is a cogent reason for early recognition of the condition, but even in early cases there are many that do not terminate so happily but continue to progress toward an acute development of hyperthyroidism. Again it is often found that the rest cure is only temporary and that as soon as activity is resumed the serious symptoms again appear. The earlier symptoms may not be such as to disturb the patient and she may be thought merely nervous. In many of our cases the condition has been first noticed by the patient's friends or by the physician at a time when the patient feels perfectly well. The analogy between Graves' disease and health urged by Putnam²² is of considerable importance. He says:

The symptoms of Graves' disease shade off, if one studies a large number of cases, into the phenomena of health. The tremor, the rapid and excitable pulse,

22. Putnam: Clinical Aspects of the Internal Secretion, Tr. Cong. Am. Phys. and Surg., 1897, iv, p. 122.

the flushed skin, the restlessness of mind, are seen almost habitually in many persons who are simply called nervous, and these symptoms and more, including the staring gaze, may come on suddenly, even in a presumably healthy person. under the influence of a strong emotion, which may be called either a disease or a quasi-physiological state. . . . I will repeat in conclusion that the importance of the analogy between Graves' disease and health is urged, not as an exclusive theory, but as one profitable mode of regarding the subject.

It is, of course, impossible from the data at our disposal to determine the precise value of the serum in the treatment of hyperthyroidism. I believe that as far as numbers are concerned an equally large series may be shown which have done as well or perhaps better on purely medical treatment, but the type of case counts for everything. Practically all of the first 100 patients and at least 75 per cent. of the remainder have used the serum only as a last resort after having tried rest, tonics, thyroidectin, and a great variety of medical measures without relief. In many such cases when the gamut of general therapy has been run the serum has been given with the most striking and immediate benefit. In the majority of cases, however, the improvement, although positive and undoubted, has been slow and gradual, perhaps requiring six to eighteen months for the final complete recovery. This is not in any way unusual, since recovery after surgical removal of the gland is in many cases slow, requiring one or two years.

CONCLUSIONS.

This work is the first attempt to treat disease in the human subject by means of a specific cytotoxic serum, and our own conclusions, subject to revision as experience increases, are as follows:

1. The serum has a specific effect in neutralizing the toxic action of the thyroid secretion.
2. As a therapeutic agent it gives results which can not, in many cases, be attained by any other medical means.
3. Not all cases presenting symptoms of thyroidism can be treated successfully with serum, because not all cases are purely hypertrophied in origin.
4. The rapid amelioration of symptoms in the acute toxic cases, similar in most respects to the well accepted instances of neutralization of toxin by antitoxin, is a weighty argument in favor of believing the symptoms to be due to the toxic effects of hyperthyroidism.
5. The beneficial results of combined treatment, especially in the older cases, indicates a *dysthyroidism* as well as *hyperthyroidism* as a factor in the production of symptoms.

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TRANSFUSION OF BLOOD IN THE TRANSPLANTABLE
LYMPHOSARCOMA OF DOGS.*

GEO. W. CRILE AND S. P. BEEBE.

No phase of experimental cancer research has aroused more interest than the demonstration of an immunity subsequent to the spontaneous recovery from implanted tumors. The truth of this principle has been established by numerous observations in various laboratories during the last three years, and yet there are a few observations in the older literature on the spontaneous retrogression of experimentally inoculated tumors. Wehr¹ in 1883 noted the spontaneous retrogression of tumors developed by transplanting fragments of a medullary carcinoma in dogs; Smith² and Washbourn in 1897 noted the spontaneous retrogression of tumors developed by planting fragments of a round celled sarcoma found on the sexual organs of dogs, and they made the further very noteworthy observation that after spontaneous healing of the tumors there existed an immunity which protected against further implantation; Leo Loeb³ in 1902 noted retrogression of many of his transplantable sarcoma in rats; Sticker⁴ in 1904 confirmed Smith and Washbourn's observations on the spontaneous retrogression of the sarcoma in dogs. It is, however, only within the last three years since the transplantation of carcinoma and sarcoma in mice and rats that the importance of this retrogression in the tumor process has been appreciated. The authentic demonstration that these transplantable mouse carcinoma retrogress

* The laboratory work forming the basis for this report was done at the Loomis Laboratory of Cornell University Medical College, under the auspices of the Huntington Fund for Cancer Research of the General Memorial Hospital. Received for publication March 24, 1908.

spontaneously in a varying percentage of cases and that subsequently there exists an immunity sufficient to protect the animal against further inoculation of like virulence is one of the fruits of American cancer research, and although this work of Gaylord and Clowes⁵ was at first rejected by some investigators, notably Bashford⁶ and Ehrlich,⁷ it is now accepted everywhere as one of the foundation principles in cancer investigation. These results with the additional demonstration by the same investigators that the serum of the spontaneously recovered animals contains some substances capable of inhibiting the growth of the tumor, have led directly to the present experiments.

The possibilities of a serum therapy for malignant tumors have been the subject of experimental investigation in many laboratories and we have lively hopes that some procedure which will be in accord with the method by which nature inhibits the process and finally absorbs the tumor mass may be applied therapeutically. In this relation the most important facts regarding immunity, which have been determined by the experimental work of the last five years, are the following:

First. A certain percentage of animals are naturally immune to tumor implantation.

Second. A certain percentage of those animals which can be successfully implanted recover spontaneously, and the tumors are completely absorbed. (The percentage of these two groups depends largely upon the virulence of the tumor.)

Third. The spontaneously recovered animal may not be successfully implanted a second time with tumors of like virulence.

Fourth.* There is no known method of rendering a susceptible animal immune except by the actual growth and subsequent retrogression of the tumor.

The nature of the immunity possessed by the spontaneously recovered animal or by the naturally immune animal is at

* During the last year several reports have appeared dealing with immunity. It is probable that various methods may suffice to immunize an animal against tumor implantation, such as inoculations with organ and embryonic tissue extracts.

present a subject for active discussion. It does not seem to correspond exactly to the immunity developed toward infections, and yet the serum of the recovered animal seems to exert a harmful action on living tumor cells. It has been repeatedly observed that any influence such as unfavorable hygienic surrounding, unsuitable food, repeated hemorrhages which diminish the general resistance also decrease the immune forces opposing the tumor growth.

Ehrlich's⁷ idea that tumor growth is a matter of suitable food-stuff, or if not food-stuff directly then some intermediate group which permits the tumor cells to make use of the circulating food-stuff already present, is an ingenious attempt to explain the process, but it fails to account for the many varied facts already discovered. A further discussion of the relation of his theory to the present work will be given later in the paper.

The demonstration by Gaylord and Clowes⁸ that the serum of the immune animal had an injurious effect upon tumor cells, and that in some instances it seemed to aid in the inhibition of a growth already implanted, led us to try the effect of a direct transfusion of the whole blood of a spontaneously recovered and therefore immune animal to an animal with actively-growing tumors. The method was one previously employed by one of us⁹ in a large series of transfusions and demonstrated to be, in itself, an absolutely safe operative procedure if done with proper technic.

TECHNIC. — The transference of the blood is accomplished by the following technic: an artery, preferably the carotid, is exposed from its bifurcation well down to the root of the neck. The artery is ligated at its bifurcation. A special artery clamp is adjusted upon the artery near the root of the neck. By an adjustable screw clamp the lumen of the vessel may be closed without damage to the intima, thus preventing clotting of blood. The artery is severed with a sharp scissors near the ligature. The adventitia is drawn well through over its end and divided, thus leaving the free end of the artery for manipulation. The external jugular

vein of the recipient is likewise freed by dissection as far as convenient. The distal part is then ligated and on the proximal end a similar adjustable clamp is placed, closing its lumen. The vein is then divided with a sharp scissors as in the case of the artery, the adventitia is drawn out and snipped off close to the divided end of the other coats. The end of the vein is now pushed through the special transfusion cannula. The end of the vessel thus pushed through is now turned back as a cuff over the cannula and held fast by a fine silk or linen ligature, tied in the second groove. The animals are now approximated so that the artery and the vein will come in contact with each other. By means of a hemostat, grasping the handle of the cannula, and with two or three very small forceps grasping the divided end of the artery, the artery is drawn over the cannula and held in place with a firm tie of a small silk or linen ligature in the first groove. The adjustable clamp is removed from the vein, then from the artery. At once a stream of blood rushes across the anastomosis, filling the vein.

Since this technic unites the circulation of the two animals in such a manner that intima only comes in contact with intima, the blood stream, therefore, comes in contact with nothing but intima and clotting does not follow. The full head on stream from the artery would, if not guarded, in some instances at least, overwhelm the heart. It is necessary to control the rate of flow by pressure upon either the artery or the vein. In this manner the rate of flow and the amount transferred is under immediate control.

We aimed at transferring as much blood as possible; the mucous membrane, the conjunctiva, even the skin of the recipient became red as the transfusion progressed. In a few instances urticaria was noted on the abdomen.

Transfusion is most readily accomplished by the use of a special cannula for this purpose. This cannula is a tube having a short projecting handle for facilitating manipulation, and two grooves in the outer surface of the cylinder.

The experiments forming the basis of this report were

made upon a series of dogs affected with a transplantable lymphosarcoma.* Such a tumor has been the subject of observation and experimental transplantation by a number of investigators, the first publication, that of Novinsky,¹⁰ having appeared in 1877. Wehr¹¹ in 1883, Duploy and Cazin¹² in 1894, Geissler¹³ in 1895, Smith and Washbourn¹⁴ in 1897, Powell White¹⁵ in 1892, Sanfelice¹⁶ in 1904, Sticker¹⁷ in 1904, Bashford, Murray and Cramer¹⁸ in 1905, and Beebe and Ewing¹⁹ in 1906 have described tumors found upon the genital organs of dogs. In most instances there have been observations upon the transmission of these tumors by coitus, but the histological diagnosis of the different observers has not been identical. However, as one reads the description of the process, one is more and more convinced that these men have all been dealing with the same type of growth, and that it is similar to the tumors which have been under observation in this laboratory during the last two years.

There is a difference of opinion among the various investigators who have studied the process as to whether it is a real tumor. Wehr and Geissler call it carcinoma, Novinsky, Smith and Washbourn, Sanfelice, Sticker, Beebe and Ewing agree that the tumor is a sarcoma, while Powell White calls it a contagious growth; and Bashford, Murray and Cramer are positive that the facts can only be explained by calling it an infectious granuloma. The controversy, therefore, is between those who believe the process an infectious granuloma and those who believe it to be a true tumor. The dispute may not be settled by the histological examination, for Bashford, Murray and Cramer agree that the histological appearance, the local mode of origin, its mode of growth from its own elements, the impossibility of transferring it to other species of animals are tumor attributes. The chief arguments by which they controvert the tumor nature of the growths are the following:

* Various investigators who have studied this process have differing opinions regarding its nature, and it seems best, therefore, to examine this matter in some detail before proceeding to a description of the transfusion.

First. The infectious character of the process, as evidenced by its mode of transmission, *i.e.*, coitus.

Second. Necrosis of tumor grafts and development of the new tumor from the surrounding connective tissue cells of the host.

Third. Identity of transplantation experiments with the processes which arise after injection of tubercle bacilli.

Fourth. The more frequent occurrence of the tumors in young adult dogs and their comparative infrequency in older animals.

It is our purpose to examine critically these arguments and state our own position with reference to them. With regard to the mode of transmission, it is a matter of common observation that coitus in the dog is a peculiar act, and one that is in most cases accompanied by some abrasions of mucous membrane. If, in addition, either animal has an ulcerating tumor on the genital organs, the possibilities are very great of infecting the other by a transplantation of tumor cell to abraded surface. In our laboratory we have repeatedly shown that it is only necessary to rub a freshly-cut tumor over a raw surface in order to secure subsequent growth of the neoplasm. It is worth while to note beside that this tumor has in the course of nature been subjected to repeated transplantation in such manner, and as we know from the recent laboratory work, the transplantation of a tumor through a series of susceptible hosts raises its virulence, so we should expect this tumor to be more readily transferred than others which have not had such a history. The mode of transmission of the tumor is the result of a somewhat unusual combination of factors which are not incompatible with the acceptance of the process as a neoplasm.

The second argument is of much greater significance than the first. If it is true that a graft of the tumor when implanted in a new host suffers complete necrosis while a new process grows from the surrounding connective tissue cells of the host, then we are surely dealing with an infection and not a tumor. On this point we have the experimental

evidence of Beebe and Ewing as follows: When a graft of the freshly-cut tumor is implanted subcutaneously in a new host, the larger portion of the graft necroses, but there remains a layer of living cells about the periphery of the piece sharply separated in many cases from the tissue of the host, and it is from these cells that the new tumor develops. These experiments have been made a number of times, and there is no doubt of the interpretation of the findings; moreover, Bashford, Murray and Cramer admit that in more rapidly-growing tumors the chief part of the growth arises from the tumor cells, and the transformation of the fibroblasts is not always in evidence. When the small wound made by transplanting the graft becomes infected, the whole series of events is obscured, and the relations of the cells of the host in the formation of the tumor may be very uncertain. When metastases of the tumor are found in the liver, lung, and kidney the cells are sharply separated from the liver tissues, and in no case can we find any evidence of a gradual metamorphosis of host cell to tumor cell. The behavior of tumor grafts when transplanted affords the most conclusive evidence regarding the nature of the suspected tumor, and in accepting the results of Beebe and Ewing, which are in direct contradiction to those of Bashford, Murray and Cramer, we find that this method of experimentation demonstrates the tumor character of the process.

The third argument in opposition is answered by the experiments of Beebe and Ewing just quoted. There is in these transplantation experiments no evidence of a similarity between the processes reactive to tumor transplantation and to injection of tubercle bacilli.

Fourth, the tumors are not found exclusively upon young animals. Many older animals show the tumor, and in most instances it pursues a more malignant course in the older animals. The method of transmission favors its occurrence in larger numbers in young, adult, sexually active animals, and it is a well-recognized clinical observation that sarcoma is more commonly found in young individuals and carcinoma in those of advanced age.

A critical examination of the objections of Bashford, Murray and Cramer reveals, therefore, nothing which is not compatible with the tumor idea and which is not best explained and understood by considering the process a neoplasm. There is, in addition, a considerable amount of collateral evidence which points in the same direction, viz.: the tumor produces general metastases, which lead to a fatal issue; no method of infection in which anything less than a living cell has been transferred from one host to another has ever given tumor growth. The range of temperature to which one may subject the tissue and still have growth indicates that the critical points are those of a highly developed protoplasm and not a microörganism. No microörganisms have been found which bear any relation to etiology, although Ewing²⁰ has studied a large number of the artificially transplanted as well as primary tumors. In a few instances spiral organisms have been found on the ulcerated surfaces of some of the tumors, but in no case in non-ulcerated tumors. Even in the ulcerated growths the spiral forms are found only a short distance from the surface, and it is now well known that such organisms are abundant on most infected, ulcerating surfaces, and their presence has no etiological relation whatever to the process under discussion. From the total available evidence we are convinced that the process is a true tumor.

In the following pages we give the actual details of each transfusion, and by comparing these with the diagrams of the tumors we believe an accurate idea may be gained of the course of events in each experiment. Following these experimental records we propose to discuss their significance in regard to tumor immunity. The tumor animals had in every case been kept under close observation for some weeks prior to the transfusion, and only those whose tumors had never shown any tendency toward spontaneous regression were chosen as recipients for the transfusion.

DETAILS OF TRANSFUSION.

FIRST EXPERIMENT. — *Recipient.* Dog No. 133, long-haired mongrel, weight seventeen kilos. Planted December 6 in the same lot with Dog

No. 125. Four tumors developed and grew slowly at first but more rapidly later, and at the time of the transfusion they were in a flourishing condition.

Donor. Dog No. 289. A strong healthy animal weighing nineteen kilos, in excellent physical condition. He had been planted previously in a group of six dogs that gave fifty-five per cent growth, but he failed to take the tumor. This donor was therefore naturally immune.

Transfusion. March 20, Dog No. 133 bled six hundred cubic centimeters and transfused with one thousand five hundred cubic centimeters from Dog No. 289. In five days following the transfusion the tumors of Dog No. 133 began to regress and the absorption continued steadily (see charts) until the tumors had been entirely absorbed. The animal was replanted with the tumor on June 11 and again on August 28 without growth.

SECOND EXPERIMENT. — *Recipient.* Dog No. 125, medium-sized, curly-haired mongrel, weight about fourteen kilos. Planted on December 6 by trocar; four abdominal tumors developed by January 17 and continued to grow rapidly. On February 23 tumor No. 3 was partially removed and plants were made from it into the back of the same animal.

Donor. Dog No. 163, short-haired mongrel, weight about sixteen kilos. Had grown tumors six months before but they had completely regressed except a small nodule on the abdomen which remained stationary for some weeks. To determine whether the animal was yet immune he was planted on December 14. No tumors developed but the old nodule increased in size for two weeks and then completely regressed. The animal had no tumors on March 20.

Transfusion No. 1. March 20, five hundred cubic centimeters of blood were withdrawn from the femoral artery of the recipient, and the same quantity put into his femoral vein from the donor, Dog No. 163.

Regressive changes were seen in the tumors of Dog No. 125 on March 25 and by following the charts it will be seen that this regression continued steadily until the tumors on the abdomen had been completely absorbed, the time required being about eight weeks.

On April 20 a metastasis appeared in the right groin (see chart) and on April 12, May 1, and May 16 the tumors previously planted on the back appeared and began to grow. Because of this growth it was determined to do a second transfusion using as a donor Dog No. 133, the animal cured by the previous transfusion.

Transfusion No. 2. May 21, Dog No. 125 bled from the jugular but through an error the amount of blood was not measured; five hundred and fifty cubic centimeters of blood were transfused from Dog No. 133.

Following the transfusion the tumors regressed for a time and then became stationary. Later they began to grow very slowly so that it was determined to do a third transfusion.

Transfusion No. 3. The donor, Dog No. 278, had been cured by a previous transfusion (see experiment No. 4 A). August 29, Dog No.

125 bled six hundred cubic centimeters and transfused with three hundred cubic centimeters. We intended to make a much larger transfusion and were disappointed in the amount actually transferred. The tumors continued to grow very slowly. A fourth transfusion was determined.

Transfusion No. 4. Donor was Dog No. 491, animal of about thirteen kilos body weight that had previously grown the tumors, but had spontaneously absorbed them. Dog No. 125 was bled six hundred cubic centimeters and transfused with five hundred and fifty cubic centimeters from Dog No. 491. Five days later Dog No. 125 had a secondary hemorrhage at the seat of operation and was so weakened by it that he died.

THIRD EXPERIMENT. — Recipient. Dog No. 116, black and tan, weight eight kilos. This animal was in very poor physical condition at the time of the transfusion. Tumors were planted on January 7, first growth of tumors noticed on February 13. The mange developed in the animal about January 30, and the disease, together with the increasing growth of the tumors, had made the animal very cachectic.

Donor. Dog No. 244, fox terrier, weight, nine kilos. This animal was not in good physical condition at the time of the transfusion. He had grown four small tumors previously and they had completely regressed. Following the transfusion, Dog No. 244 was again planted with the tumor with positive results in each plant, and metastases formed in a few weeks. Therefore the immunity which he possessed at the time of the transfusion must have been very weak. Dog No. 244 will be referred to in a later transfusion.

Transfusion. March 20, Dog No. 116 was bled four hundred cubic centimeters and received six hundred cubic centimeters from Dog No. 244. The tumors of Dog No. 116 were not affected by the transfusion; they continued to grow steadily and the animal died on April 17 in a very cachectic condition.

FOURTH EXPERIMENT, A. — Recipient. Dog No. 278, mongrel, weight fifteen and one-half kilos. Planted on February 2. Two tumors appeared on March 7, and the remaining two on March 28. At the time of the transfusion he had four good tumors which had been growing rapidly.

Donor. Large vigorous normal animal which had never grown tumors was used as a donor. He had been received at the laboratory on the day previous to the transfusion.

Transfusion. May 21, Dog No. 278 was bled four hundred and thirty cubic centimeters of blood and received one thousand five hundred cubic centimeters from his donor. Following the transfusion his tumors rapidly regressed. All the tumor tissue was absorbed by July 20. The animal was replanted on August 2 and again on August 28, but there was no growth from these grafts.

FOURTH EXPERIMENT, B. — Donor. As a part of the same experiment the blood from Dog No. 278, the recipient in the previous transfusion, was passed into Dog No. 115.

Recipient. Dog No. 115. Planted on January 7 with negative results. The animal was then bled from the carotid in order to reduce his resistance, and following this hemorrhage he was again planted with tumors on February 14. The plants all gave positive growth, but after reaching the size of a hickory nut they began to regress.

Transfusion. The transfusion was done in this case to determine whether the blood from a dog (No. 278) growing the tumors rapidly would stop the regression if transfused into an animal with regressing tumors (Dog No. 115). Dog No. 115 was bled two hundred and thirty cubic centimeters and transfused with four hundred and thirty cubic centimeters from Dog No. 278. The tumors of Dog No. 115 continued to regress though at a slower rate than before the transfusion.

FIFTH EXPERIMENT. — *Recipient.* Dog No. 132, short-haired mongrel of fifteen kilos. Planted on March 10, tumors developed April 6 and continued to grow nicely until the day of the transfusion.

Donor. Dog No. 275. Planted on February 4. One small tumor developed, but it had been completely reabsorbed before the transfusion.

Transfusion. May 21, Dog No. 132 bled three hundred cubic centimeters and transfused with five hundred cubic centimeters from Dog No. 275. Following the transfusion the tumors in Dog No. 132 began to regress and the absorption continued steadily until the tumors were entirely gone. The animal was planted on June 11 and again on August 28 without any growth resulting.

SIXTH EXPERIMENT. — In this experiment the same animal was transfused three times on different occasions and therefore it will be necessary to describe three donors.

Recipient. Dog No. 137, short-haired mongrel, weight seven kilos. Planted on March 2. Tumors first appeared on April 6 and continued to grow steadily. (See charts.)

Donor No. 1. Dog No. 157, mongrel, weight nine and one-half kilos. This animal had been planted in the same group with Dog No. 137 but failed to grow the tumors and was therefore supposed to be naturally immune.

Donor No. 2. Dog No. 282, white bull-dog, ten kilos. Had been planted on January 30, and again April 1, with negative results in each case, and was therefore supposed to be naturally immune.

Donor No. 3. Dog No. 274, setter, of seventeen kilos body weight. The animal was planted on February 4 with positive results, four tumors developing and growing to a considerable size but had later retrogressed and only very small fragments were left of the tumors at the time of the transfusion.

Transfusion No. 1. May 3, Dog No. 137 was bled one hundred and forty cubic centimeters and received four hundred and fifty cubic centimeters from Dog No. 157. As a result of the transfusion tumors of Dog No. 137 became somewhat softer but they continued to grow. (See charts.)

Transfusion No. 2. Since May 3 Dog No. 137 had lost one-half kilo in weight and was in poorer physical condition generally. May 21 Dog No. 137 was bled two hundred and twenty-five cubic centimeters and received four hundred and twenty-five cubic centimeters from Dog No. 282. The tumors continued to grow.

Transfusion No. 3. On June 10, Dog No. 137 weighed one and one-half kilos more than on the date of the last transfusion May 21. The general condition had not improved with this increase in weight and the tumors had continued to grow. It seemed best to do a much larger transfusion. On the date given, June 10, Dog No. 137 was bled five hundred cubic centimeters and given seven hundred and fifty cubic centimeters from Dog No. 274. Following this transfusion Dog No. 137 improved very markedly in physical condition and the tumors began to regress. On September 24 the last fragment of tumor had been absorbed. This animal was replanted with tumors August 28, but no growth resulted.

SEVENTH EXPERIMENT. — *Recipient.* Dog No. 199, weight nine kilos. Planted on November 3 with negative results. Planted a second time on December 14. Growth of plants first apparent on January 17. On March 23, April 1, and April 12 small pieces were removed to serve as seed for additional plants in other animals. On May 3, the date of the first transfusion, Dog No. 199 had four tumors which had been growing rapidly during the three weeks immediately preceding. The animal was in fair condition.

Donor. Dog No. 273, mongrel setter, weight twelve kilos. Planted on February 4. Growth on March 7, continued until April 10; at the time of the transfusion two nodules about the size of a grain of wheat remained to be absorbed. Dog in good condition.

Transfusion No. 1. May 3, Dog No. 199 was bled one hundred and fifty cubic centimeters and transfused with five hundred and fifty cubic centimeters of blood from Dog No. 273. Following the transfusion Dog No. 199 improved in general condition and gained one kilo in weight during the following two weeks, but the tumors continued to grow.

Donor No. 2. Dog No. 271, weight 13.5 kilos. Was planted on February 4 with negative results and again on April 1 with the result that two small tumors developed. They grew to the size of a hazel-nut but were completely reabsorbed on May 13. Dog in fine condition.

Transfusion No. 2. May 21, Dog No. 199 was bled two hundred and twenty cubic centimeters and immediately transfused with seven hundred cubic centimeters of blood from Dog No. 271. Following the transfusion the tumors did not grow larger, but they did not begin to regress markedly until three weeks later, at which time it was evident that the tumors were being absorbed. Regression continued steadily until the tumors had been completely absorbed, September 19. Replanted on November 2 without growth.

EIGHTH EXPERIMENT. — *Recipient.* Dog No. 244, fox terrier, weight

eight and one-half kilos. This animal has been referred to in Experiment No. 3. Following his use as a donor in that experiment he was replanted with the tumor March 23. Three of the four plants gave growth on or before May 13, and the general condition of the animal became progressively worse. When the tumors had reached the size of hickory-nuts they were treated with various bacterial toxins by Dr. Tracy, but the results in this case were negative and it was therefore determined to resort to transfusion.

Donor No. 1. Dog No. 123, weight, ten kilos, was planted with tumors on December 14. Visible growth appeared on January 10, and continued nicely until January 30, on which date toxin treatment was begun by Dr. Tracy. The treatment resulted in a complete cure of the tumors. On May 4 and again on July 11 the animal was replanted with tumor grafts, but no growth followed in either case and our conclusion was that the animal must possess some degree of immunity.

Donor No. 2. Dog No. 132, an animal previously cured by transfusion. (See fifth experiment.)

Transfusion No. 1. July 31, Dog No. 244 was bled three hundred and twenty-five cubic centimeters and immediately transfused five hundred and fifty cubic centimeters of blood from Dog No. 123. The transfusion did not have much effect upon the tumors, however, as they continued to grow and the animal grew worse in general physical condition.

Transfusion No. 2. August 26, at the time of the second transfusion, Dog No. 224 was very cachectic. He was bled two hundred and twenty-five cubic centimeters and transfused from Dog No. 132. Unfortunately, through the mistake of an assistant, Dog No. 132 was not weighed immediately before the transfusion, so that we do not know exactly how much blood was taken from the donor. It was a satisfactory transfusion, however.

In spite of a marked hemolysis following the transfusion, Dog No. 244 gained in weight, his general physical condition improved somewhat, and the tumors soon began to regress. Although the tumors continued to regress (see charts), his general physical condition did not remain good. He gradually grew cachectic and died October 14. At the autopsy it was found that the axillary, retroperitoneal and mesenteric lymph nodes were enlarged. One of the retroperitoneal glands contained metastatic tumor growth, the others failed to show it.

NINTH EXPERIMENT. — Recipient. Dog No. 444, mongrel pointer, seven kilos. Planted on June 11, tumor growth apparent on July 12, and the growth continued steadily until the date of transfusion.

Donor. Dog No. 435, weight nine kilos. Planted on April 20 with positive results, three out of four plants showing positive growth, but they never grew to large size and soon began to regress. The process was complete on August 8, and on the date of transfusion the animal was in excellent condition.

Transfusion. August 27, Dog No. 444 was bled three hundred and

thirty-five cubic centimeters, and received four hundred and fifty cubic centimeters of blood from Dog No. 435. The tumors showed evidences of regression in a few days after the operation, and the process continued steadily, our chart showing complete absorption on September 30. The animal was replanted November 9 and again on September 4 without resulting growth.

TENTH EXPERIMENT. — *Recipient.* Dog No. 406, mongrel setter, weight fourteen kilos. Planted March 23. Growth visible on April 18, and by May 20 four tumors had developed and were growing nicely.

Donor No. 1. The same animal which was used in the fourth experiment. He had lost three kilos in weight during the three weeks since his previous operation, and was in much poorer physical condition.

Donor No. 2. Dog No. 269, a large healthy animal weighing 20 kilos. Tumors had been planted in this animal January 18; the first growth appeared February 6 and continued very slowly until April 12. Regression began at the later date and continued very slowly to a complete absorption.

Transfusion No. 1. June 10, Dog No. 406 was bled five hundred and fifty cubic centimeters and transfused with one thousand two hundred and fifty cubic centimeters from donor No. 1. The tumors did not regress as a result of this transfusion, nor did the general condition improve.

Transfusion No. 2. July 15, Dog No. 406 was bled seven hundred cubic centimeters and received eight hundred cubic centimeters from the donor, Dog No. 269. Following this transfusion, the tumors began to regress immediately, and the general condition of the dog improved. The absorption of the tumors was complete on August 17.

SUMMARY OF TRANSFUSION EXPERIMENTS.

No.	Donor's Immunity.	Recipient.		Transfusion.		Result.
		Weight.	Condition of Tumors.	cc. Bled.	cc. Transfused.	
I.	Natural immunity.	17 kg.	Growing well.	600	1,500	Complete absorption of tumors.
II.	I. Spontaneous recovery.	14 kg.	Growing rapidly.	500	500	Complete absorption of the first set of plants.
	II. Transfused recovery.	14 kg.	Growing slowly (second set).	550	Regression at first, growth later.
	III. Transfused recovery.	14 kg.	Growing slowly (second set).	600	300	Tumors continued to grow slowly
	IV. Spontaneous recovery.	14 kg.	Growing slowly (second set).	600	550	Death from secondary hemorrhage five days later.
III.	Supposed spontaneous recovery.	74 kg.	Growing rapidly.	400	600	Negative. Tumors continued to grow. Dog died a month later.
IV.	A. Unknown.	154 kg.	Large, rapidly growing.	430	1,500	Complete absorption of tumors.
	B. None.	12 kg.	Regressing slowly.	230	430	Tumors continued to regress, but more slowly.
V.	Spontaneous recovery.	15 kg.	Growing well.	300	500	Complete absorption.

SUMMARY OF TRANSFUSION EXPERIMENTS. — *Continued.*

No.	Donor's Immunity.	Recipient.		Transfusion.		Result.
		Weight.	Condition of Tumors.	cc. Bled.	cc. Transfused.	
VI.	I. Naturally immune.	7 kg.	Growing steadily.	140	450	Tumors became somewhat softer, but they continued to grow.
	II. Naturally immune.	7 kg.	Growing steadily.	225	425	No effect.
	III. Spontaneous recovery.	7 kg.	Growing steadily.	500	750	Complete absorption.
VII.	I. Spontaneous recovery.	9 kg.	Growing rapidly.	150	550	General condition improved. Tumors continued to grow.
	II. Spontaneous recovery.	9 kg.	Growing rapidly.	220	700	Complete absorption.
VIII.	I. Cured by toxins.	8 kg.	Tumors growing well.	325	550	No effect.
	II. Transfusion recovery.	8 kg.	Very cachectic.	225	Almost complete absorption.
IX.	Spontaneous recovery.	7 kg.	Steady growth.	335	450	Complete absorption.
X.	I. Same dog as Exp. IV., A.	14 kg.	Steady growth.	550	1,250	No effect.
	II. Spontaneous recovery.	14 kg.	Steady growth.	700	800	Complete absorption.

From the summary we see that of the ten animals which were treated by this method of transfusion, seven were completely cured, in two of the remaining there was very marked effect from the transfusion, and only one died without showing any regression as a result of the exchange of blood. In this latter case there is more than a reasonable doubt regarding the immune condition of the donor, since the implantation of tumor grafts following the transfusion resulted in positive growth and subsequent development of large tumors.

Having determined the fact that a large transfusion of blood from an immune animal to an animal with growing tumors is followed by their regression and complete absorption the questions at once arise:

1. By what mechanism is the result accomplished?
2. What is the value of different sorts of blood in this reaction?
3. What conditions of transfusion are best suited to give a favorable outcome?

We must say frankly that we do not know how the blood has caused the results noted. It is possible to maintain various arguments on this point, but it is our purpose in this paper to put the facts on record rather than to theorize on the various possibilities in tumor immunity. We shall, however, call attention to a few obvious relations which our work has to that of other investigators. Those who believe in atreptic immunity as outlined by Ehrlich will be inclined to argue that by the exchange of blood we have deprived the tumor of its specific food-stuff, and so it has merely died of inanition. Such a conclusion implies that the blood has negative action only, and that immunity is established by the exhaustion of specific food-stuff. The objections to the acceptance of such a hypothesis will become apparent as we proceed.

The histological picture of the regressing tumors is nearly the same as that of tumors regressing spontaneously, but in a few cases active degenerations were found in tumors regressing after transfusion which have no analogy in the

spontaneously regressing tumors that we have studied. There is practically no evidence that the blood acts directly as a lysin, certainly not a lysin of a high degree of activity. The action is spread over so long a time that highly active agents are practically ruled out. Until we know more of the nature of immunity to tumors we can only say that these experiments indicate that the blood of the immune dog contains some expression of this immunity of sufficient potency to influence the course of tumor growth exposed to its action; perhaps by stimulating the general nutritive processes, for we see improvement in general physical condition following transfusion. We know, too, that the blood of the tumor animal is hemolytic and perhaps generally toxic, so that the removal of such an injurious circulating medium and its replacement by the blood of a vigorous animal may possibly stimulate the latent defence of the tumor subject.

On the basis of so few experiments, we cannot decide positively the relative value of blood from normal animals as compared with that of spontaneously recovered animals. Our impression is that the blood from the recovered animal is somewhat better than the normal blood, but the quantity of the transfusion as well as its quality are of value in determining the outcome.

In this connection we call attention to Experiments IV. and X. The same donor was used for the first transfusion in each case. At the time the transfusion in Experiment IV. was done the animal was in a strong, healthy condition. The transfusion in this instance was followed by complete absorption of the recipient's tumors. The donor lost weight and his general physical condition became progressively worse in the interval between Experiment IV. and Experiment X. The operative procedure in Experiment X. was successful in every way, a large volume of blood being transferred, but no effect was produced upon the tumors. It seems probable that the loss of blood in the first transfusion, together with the generally unfavorable physical condition at the time of the second experiment, left him in a poorer condition as regards tumor immunity. The second transfusion

of Experiment X., although of smaller volume, came from a spontaneously recovered dog in good condition, and it was completely successful in causing an absorption of the tumors. If the favorable result is due to the negative action of removing specific food-stuff it would seem that the first transfusion should have been successful, since it gave a more complete exchange of blood than the second. Evidently some difference in the quality of the two bloods was responsible for the regression in one case and not in the other. Further evidence of this fact is afforded by Experiment VIII. in which both donors had been cured of tumors and could not be replanted. The first transfusion from an animal cured by toxins was entirely unsuccessful, while the second from a previous transfusion recovery was followed by a nearly complete regression of the growths.

In Experiment III. we found that a spontaneously recovered animal did not give a satisfactory blood, for the tumors of the recipient continued to grow. In this case, however, we found that subsequent planting of the donor gave positive tumor growth, a condition which was not found in the other spontaneously recovered animals of our experiments.

In Experiments VI. and VII. we have evidence that the quantity of the transfusion is of some importance. In Experiment VI. the first two transfusions from naturally immune donors produced no effect on the tumors, while the first from a spontaneously recovered animal gave a much more complete exchange of blood and caused an absorption of the growths. We have no method for measuring the relative degrees of immunity possessed by the three donors further than the fact that all three would not grow the tumor, and it may be that the favorable outcome in the final transfusion was due to its quantitative aspects. If we consider that the recipient's total blood supply was twelve per cent of his body weight he had eight hundred and forty cubic centimeters circulating blood. In the first two transfusions an average of one hundred and eighty cubic centimeters of blood were removed and four hundred and thirty-seven added, so that the final volume of blood was one thousand

ninety-seven cubic centimeters, of which forty per cent came from the donor. In the third transfusion five hundred cubic centimeters were removed and seven hundred and fifty cubic centimeters added, giving a final blood volume of one thousand ninety, of which sixty-six per cent came from the donor. Following the same method of calculation we find that in Experiment VII. the first transfusion which was unsuccessful was only thirty-seven per cent, while the successful transfusion was forty-five per cent.

It appears, then, that immunity may have two factors:

First, a common factor possessed to some extent by any strong, healthy, vigorous animal, in some cases of sufficient activity to prevent successful inoculation with tumors.

Second, a specific factor which is developed by the growth and regression of tumors. What qualitative relation these two factors have to one another we cannot say on the basis of our present knowledge. Although immunity to tumors does not seem to be analogous to bacterial immunity, nevertheless these results indicate that it is to some extent a blood condition which may be transferred to another animal rendering him passively immune.

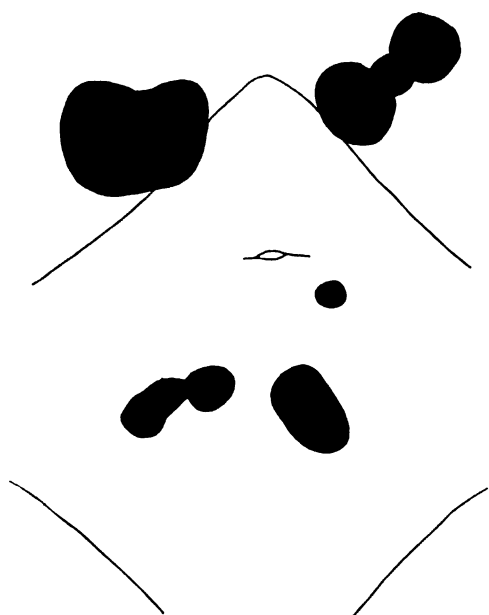
In regard to the conditions of transfusion which favor the regression of the tumors it is our opinion that the donor should be a strong healthy animal, immune to the tumor, and that a large replacement of the recipient's blood should be made giving him from twenty-five to fifty per cent more blood than is removed. Such a conclusion has its foundation in our experimental results and is what might be theoretically expected.

The possibilities for the treatment of human tumors remain to be determined

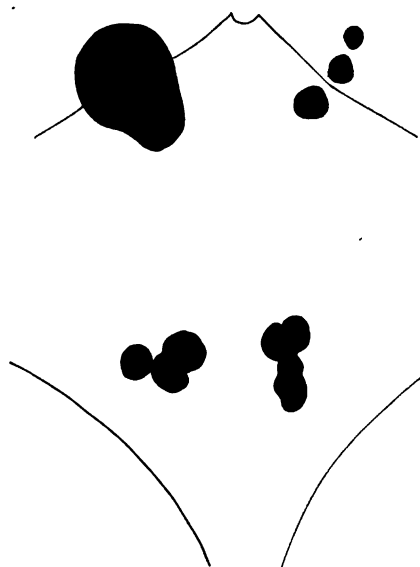
[Our thanks are due to the members of the laboratory staff who kindly assisted in the operations.]

REFERENCES.

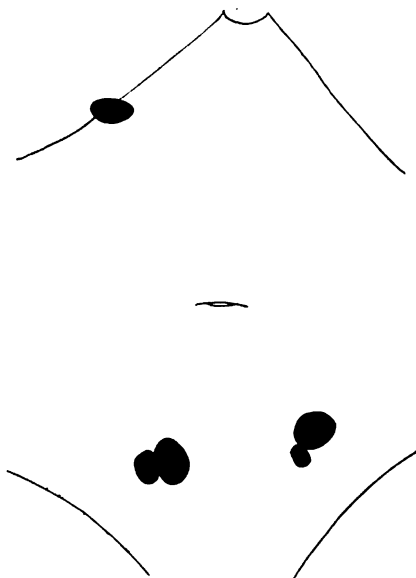
1. Wehr. *Centralblatt für Chirurgie*, No. 24, 1888.
2. Smith and Washbourn. *Transactions of the Pathological Society of London*, xlviii, 1897. *British Med. Journal*, December, 1898. *Edinburgh Med. Journal*, January, 1900.
3. Loeb. *Virchow's Archiv.*, clxvii, 1902.
4. Sticker. *Zeitschrift für Krebsforschung*, i, 1904.
5. Gaylord and Clowes. *Surgery, Gynecology, and Obstetrics*, June, 1906.
6. Bashford. *Scientific Reports on the Investigations of the Imperial Cancer Research Fund*, No. 2.
7. Ehrlich. "Experimentelle Carcinomstudien an Mäusen" *Arbeiten a.d. Kgl., Inst. f. Exp. Therapie*, 1906.
8. Clowes and Baeslack. *Medical News*, Nov. 18, 1905.
9. Crile. *Proceedings of Society for Experimental Medicine and Biology*, 1906.
10. Novinsky. Referred to in Sticker's article in *Langenbeck's Klinische Chirurgie*, lxxviii, 774.
11. Wehr. *Loc. cit.*
12. Duploy et Cazin. *Bericht des, xi, Intern. Med. Congress, Rome*, 1894.
13. Geissler. *Verhandlungen der Deutsch. Gesellschaft für Chirurgie*, xxiv, Congress, Berlin, 1895.
14. Smith and Washbourn. *Loc. cit.*
15. Powell White. *British Med. Journ.*, July, 1902.
16. Sanfelice. *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, xxxvi, 1904.
17. Sticker. *Loc. cit.*
18. Bashford, Murray and Cramer. *Loc. cit.*
19. Beebe and Ewing. *Journal of Medical Research*, xv, 1906.
20. Ewing. Unpublished research



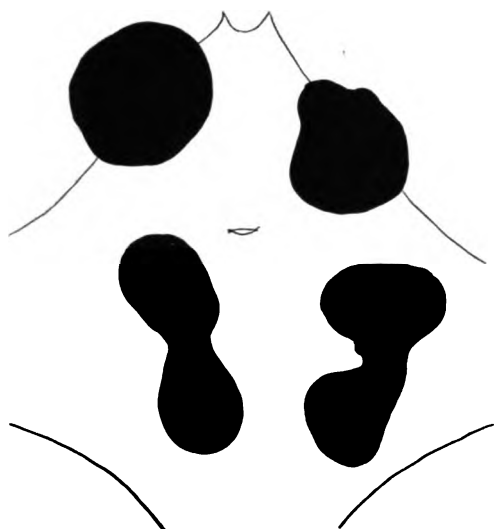
Experiment I. — Tumors on date of transfusion, March 20, 1907.



Experiment I. — April 8, 1907.



Experiment I. — April 26, 1907.



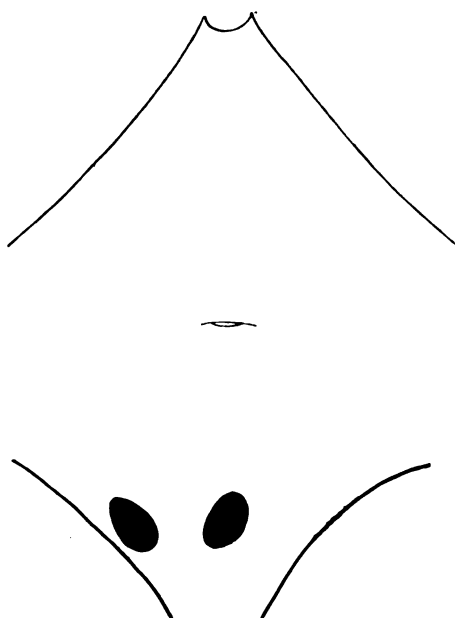
Experiment II. — Tumors on date of transfusion, March 20, 1907.



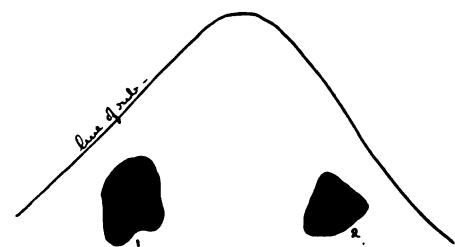
Experiment II. — Second transfusion because of growth on the back, April 18, 1907.



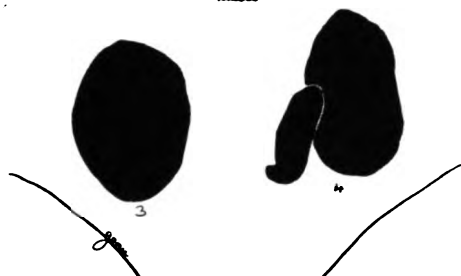
Experiment II. — May 18, 1907.



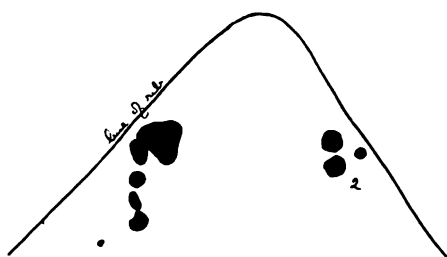
Experiment II. — July 6, 1907.



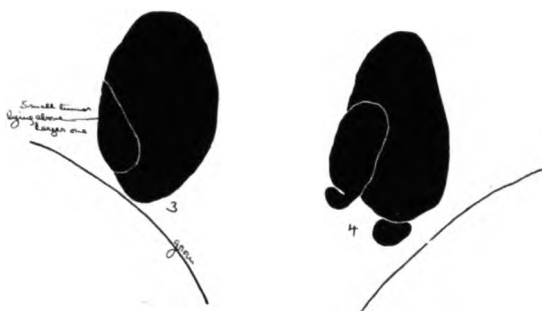
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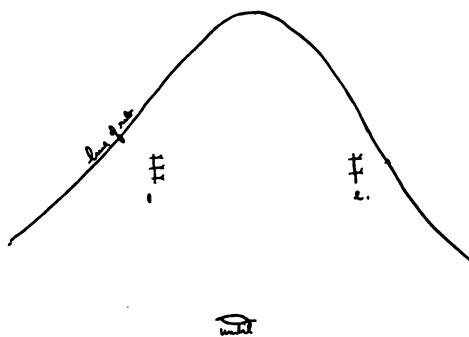
Experiment IV., A. — Tumors on date of transfusion, April 22, 1907.



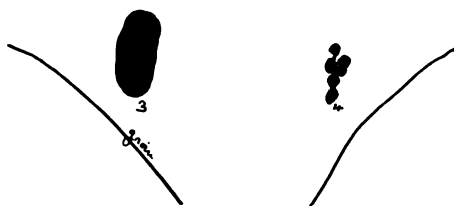
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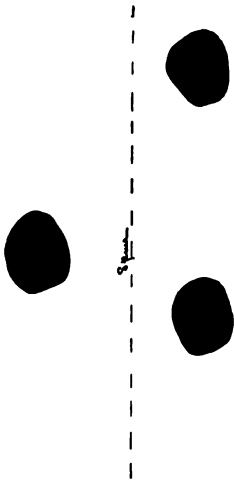
Experiment IV., A. — May 24, 1907.



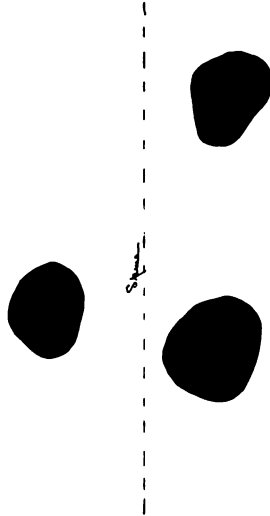
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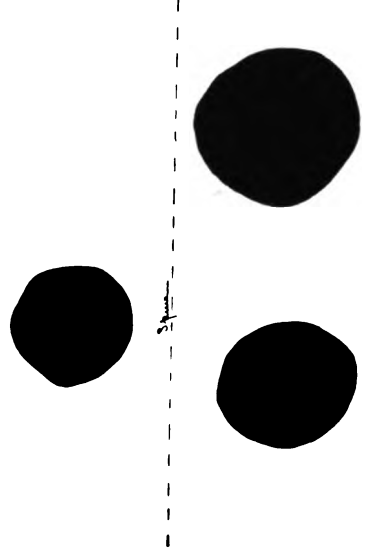
Experiment IV., A. — June 25, 1907. Two very small tumors were removed on this date for histological study.



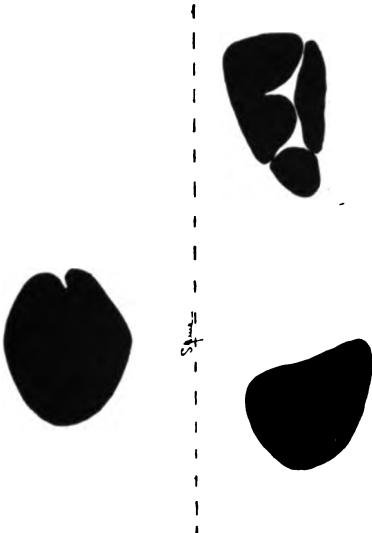
Experiment VI.— Tumors on date of first transfusion, May 3, 1907.



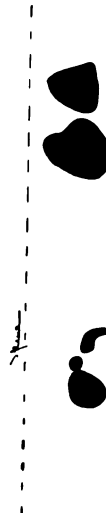
Experiment VI.— Tumors on date of second transfusion, May 21, 1907.



Experiment VI.— Tumors on date of the third transfusion, June 10, 1907.



Experiment VI.— July 2, 1907.



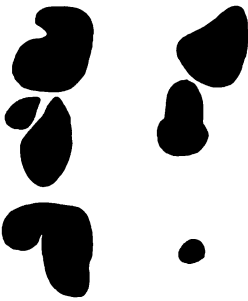
Experiment VI.— July 27, 1907. The small tumor on the left had been removed for histological study.



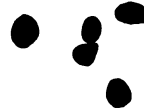
Experiment VII. — Tumors on the date of first transfusion, May 3, 1907.



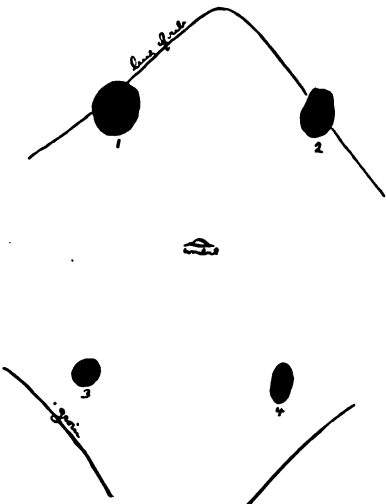
Experiment VII. — Tumors on date of the second transfusion, May 24, 1907.



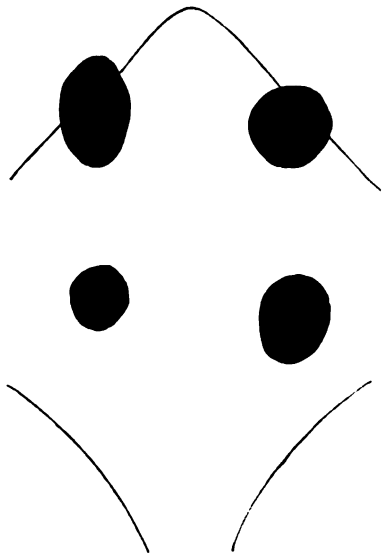
Experiment VII. — July 1, 1907.



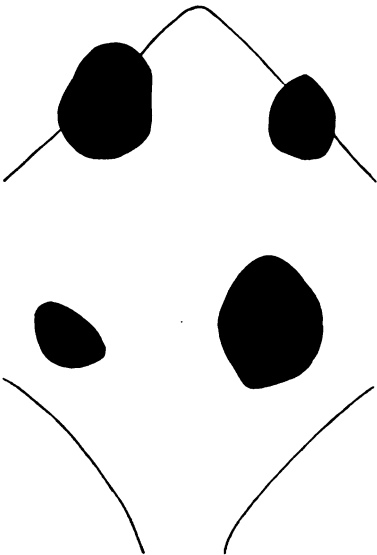
Experiment VII. — Aug. 8, 1907.



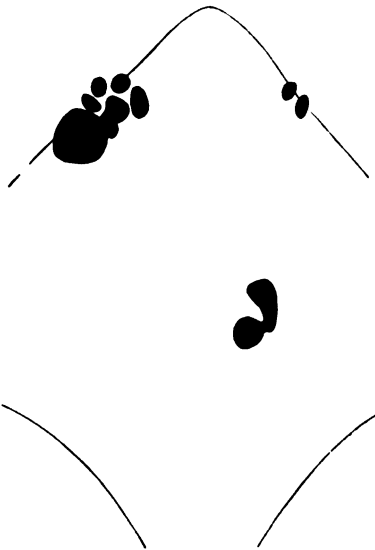
Experiment VIII. — Size of tumors on June 3, 1907.



Experiment VIII. — Tumors on date of first transfusion, July 31, 1907.



Experiment VIII. — Tumors on date of second transfusion, Aug. 26, 1907.



Experiment VIII. — Oct. 9, 1907.

**The Hemolytic Reactions of the Blood in
Dogs Affected with Transplantable
Lymphosarcoma.**

**RICHARD WEIL, M.D.
NEW YORK CITY.**

THE HEMOLYTIC REACTIONS OF THE BLOOD IN DOGS AFFECTED WITH TRANSPLANTABLE LYMPHOSARCOMA.*

RICHARD WEIL, M.D.

NEW YORK CITY.

During the course of the present year I reported a series of experiments¹ on the hemolytic properties of extracts from organs and from tumors of dogs. I showed at that time that two sorts of hemolytic substances could be extracted from tumors. One of these, which was present in undegenerated tumors, was thermolabile, and hemolytic only when activated by a substance extracted from the red blood cells. This first substance is comparable to a similar hemolytic amboceptor present in certain normal organs, e. g., kidney. At about the same time and independently it was shown by Friedmann² that a hemolytic substance could be extracted from the pancreas, which destroys the red blood cells of the same species, when complemented by some substance (probably of the lecithin group) present in the serum of certain other species. The second type of hemolysin is present only in necrotic tumors; it acts as a simple hemolysin, needing no complement. I ventured to suggest in my first paper that it is the second group of substances which probably passes into the blood, thus contributing to the anemia and the cachexia of malignant tumors.

Since that time I have had the opportunity, thanks to the kindness of Dr. S. P. Beebe, of prosecuting this investigation along somewhat different lines. I have had at my disposal dogs inoculated with the same strain of tumor previously studied, in every stage of its growth. In some the tumors were actively growing, in others they were regressing, while still others were overcoming their tumors as the result of a passive immunity conferred by transfusion. Some of the dogs were in good condition; others were cachectic to the last degree. At the same time I have had a large number of dogs not affected with these tumors, for comparison; some of the latter have been healthy, some cachectic.

It was the object of the present research to study the serum of dogs which had been inoculated with tumors, and to determine whether it possessed any of the hemolytic power characteristic of extracts made

* From the Huntington Fund for Cancer Research of the General Memorial Hospital. Laboratory work done at the Loomis Laboratory.

1. Jour. Med. Research, Boston, 1907, xvi, 287.

2. Deutsche med. Wochschr., Leipzig u. Wien, 1907, xxxiii, 585.

from the tumors themselves. In withdrawing blood from these animals and separating it into serum and corpuscles for further study, the following observation was made: It was generally found that the serum obtained from animals with tumors was stained with hemoglobin, whereas that obtained from animals not having tumors was of the normal straw color. In other words, during the process of separating out in the icebox, the serum of tumor animals had destroyed a sufficient number of its own corpuscles to give it the characteristic tinge of hemoglobin. At the same time, emulsions of corpuscles from the same animals in salt solution remained unchanged, demonstrating that the hemolysis was due not to the lowered vitality of the corpuscles, but to the hemolytic quality in the serum.

The next step was to determine the effects of definite amounts of serum both of normal and of tumor dogs on their own and on other corpuscles. For this purpose, twenty-five dogs in all were selected, of which eleven had tumors and fourteen had no tumor history. All of these dogs were treated in the same fashion. The femoral artery was laid bare under morphin anesthesia, and from twenty to thirty cubic centimeters of blood was obtained from it. The major portion of the blood was allowed to coagulate for the purpose of obtaining serum, while the rest of it was shaken up with glass beads, centrifuged, and made up into a 1 or 2 per cent. suspension in normal salt. The sera, and the corpuscles thus obtained on one day from a large number of dogs, were tested on one another on the following day. One cubic centimeter of serum was always added to an equal quantity of red cell emulsion, incubated for a few hours and transferred to the icebox, where it was allowed to remain over night; the results were noted the following morning. In this manner, 226 separate serum-corpuscle combinations were examined.

A record of one of the experiments will indicate better than any amount of description the type of result which was regularly obtained. The description of the dogs made use of in the experiment, and indicated by numbers in the table, is as follows:

Dog 455.—Four tumors growing slowly for six weeks. Condition good.

Dog 509.—Four tumors growing for two weeks very rapidly. Thin.

Dog 460.—Spontaneous recovery from tumors, which were three in number, and were very rapidly absorbed six weeks before the experiment. Very cachectic animal.

Dog 244.—Tumor almost completely absorbed, owing to repeated transfusions. Dog very cachectic.

Dog 458.—Spontaneous recovery from tumors. In fine condition.

Dogs 516, 517 and 518.—Normal.

Dog 520.—Cachectic. No tumor history.

The following table shows the comparative hemolytic effects of serum from normal and tumor dogs on corpuscles. One c.c. of serum from each animal was mixed with 1 c.c. of corpuscles of each animal, in emulsion. The record of each serum reads along the horizontal line.

CORPUSCLES.

	1-455	2-509	3-458	4-517	5-518	6-516	7-520
Serum I 455	—	—	—	v. sl.	v. sl.	v. sl.	v. sl.
Serum II-509	—	—	—	sl.	sl.	sl.	sl.
Serum III-460	+	+	+	++	++	++	++
Serum IV-244	+	++	+	+++	+++	+++	+++
Serum V-458	—	—	—	—	sl.	v. sl.	sl.
Serum VI-518	—	—	—	—	—	—	—
Serum VII-520	—	—	—	—	—	—	—

NOTE: — denotes negative reaction; v. sl. denotes very slight reaction; sl. denotes slight reaction; + denotes moderate reaction; ++ denotes strong reaction; +++ denotes complete reaction.

If the table be interpreted in the light of the preceding data, the following conclusions may be drawn from it:

1. The serum of all the tumor dogs is distinctly more hemolytic than is the serum of non-tumor dogs. Of the tumor animals, the two cachectic examples (460 and 244) are more actively hemolytic than are the other three.

2. There is a distinct difference in the resistance of the various corpuscles to the destructive activity of the sera. The corpuscles of the tumor animals are always more refractory than are the normal; they break down less rapidly and less completely.

These two phenomena, the hemolytic activity of the sera and the resistance of the corpuscles of tumor animals as compared with other dogs, dominate the entire series of experiments.

The cause of this difference has seemed to me to be a matter of some importance. It might conceivably be due to a difference in the tonicity or content in electrolytes of the serum, resulting in some obscure manner from the growth of the tumor. In order to determine this matter, each set of corpuscles was in every experiment subjected to the action, not only of a normal salt solution, but also to one of a 0.4 per cent. and one of a 1.6 per cent. strength. It would seem that the degree of anisotonicity represented by these solutions must far exceed that of any serum obtained from a living animal. Nevertheless, the activity of the resulting hemolysis was in no way comparable to that produced by the sera. The general condition of the animals can not be held

responsible, inasmuch as some of the controls were much more emaciated and cachectic than some of the tumor dogs. The hemolytic power of the serum resides, therefore, in some other factor, at present undetermined—possibly the same toxins as were extracted from the tumors in the experiments which I previously reported.

The apparent coincidence of poor general condition and marked hemolytic activity is quite striking, and might at first sight impose as a "*propter hoc*." It has, however, been pointed out that dogs in worse general condition, but without tumors, show less activity in hemolysis. On the other hand, it is perfectly justifiable to interpret the "poor condition" as a resultant of the toxic character of the circulating serum, which manifests itself in the test tube by hemolysing the contained red cells. Two exceptions occurred to the general rules above described. Dog 125, whose tumors had regressed owing to three successive transfusions, in good condition, possessed a serum of slight hemolytic power and corpuscles of slight resistance. Whether this was due to the predominance of normal blood in his vessels, I leave undecided. The second case, Dog 638, normal, has a markedly hemolytic serum, but non-resistant corpuscles. This dog had proved naturally immune to a sarcoma implantation. It may also seem contradictory that dogs which had recovered from their tumors, either spontaneously or as the result of transfusion, should still retain the hemolytic character of their serum to an excessive degree. The fact is, however, in accord with what is known of the clinical absorption of tumors. If, owing to an excessive use of x-rays or the mixed toxins, a tumor breaks down too rapidly, its absorption may lead to extreme intoxication of the organism, and even to death. Thus the disappearance of the tumor goes hand in hand with the circulation of the toxic products which have been absorbed. How long an animal may retain these products in his circulation is still to be determined.

The bearing of these experiments on the problem of the anemia accompanying malignant new growths is manifest. They demonstrate that the growth of tumors causes the development of toxic qualities in the blood serum, the exact nature of which has not been determined. It is suspected, however, to be due to the absorption of hemolytic substances previously demonstrated in the tumors themselves.

The bearing of these experiments on the practice of transfusion in curing the tumors of animals, or of men, is important. As is well known, the operation has been performed a number of times in dogs with some success. It is quite evident, however, that to mix the blood of dogs, either of which is hemolytic for the other, would entail the destruction

of a very considerable number of corpuscles, and so would, in part, defeat the very object of the transfusion. There can be no question that the test-tube reactions may be taken as a very good indicator of what would happen were the same bloods to be mixed *in vivo* by transfusion. Of this fact we have had indubitable evidence in the hemolysis resulting after transfusing the blood of one dog into the vessels of another whose serum had been shown to destroy the corpuscles of the former in test-tube experiments. (These experiments have not yet been published.) It is clear, therefore, that every transfusion should be logically preceded by test-tube reactions, and that only those animals should eventually be selected whose serum and corpuscles are mutually tolerant.

On the bearing of the experiments on the subject of human disease I must, for the present, forbear to enter. A considerable number of observations already completed go far to indicate that the results in dogs illustrate conditions which have a parallel in the blood of human cancer cases. On this important point, however, much information must be collected before final conclusions can be drawn.

My thanks are due to Dr. Beebe for his constant encouragement and help during the course of this research.

163 Eighty-sixth Street.

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American Medical Association, 103 Dearborn Ave., Chicago.

THE HEMOLYTIC REACTIONS IN CASES OF HUMAN CANCER.*

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It is the purpose of the present paper to give a brief survey of work done during the last twelve months on the subject of hemolysis in cases of human cancer. In a previous paper, on the hemolytic reactions of the serum in dogs affected with lymphosarcoma,³² it was shown that there were certain characteristic features distinguishing the serum and the corpuscles of such animals from healthy individuals of the same species. The extension of the research to the human subject was a natural corollary, and has occupied the attention of the writer more or less constantly for the past year.

The history of the problem is of considerable interest. The attempt to discover in the blood a reaction characteristic of the existence of malignant new growths is by no means new. Every fresh method of hematological research which has been developed in the laboratory has in turn been made subservient to this object. The staining methods of Ehrlich gave rise to a considerable literature, now largely forgotten, the aim of which was to demonstrate certain morphological characters of the blood cells, supposed to be pathognomonic of the presence of malignant tumors. At the present time it is generally admitted that this attempt has failed. This phase was succeeded by the study of the resistance of the red cells in various pathological conditions to anisotonic solutions of salts. As a result of these researches it has become established that the resistance of the red cells of human beings varies considerably,^{4, 22} and that it is as a rule increased in far advanced cases of malignant disease.²¹ The clinical applicability of this finding is unfortunately very slight. With the advent of the modern methods of studying

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the agglutinating and the lytic effects of serum on corpuscles, a literature of no small volume has accumulated as the result of the application of these methods to human pathology. A number of observers asserted that the serum in certain diseases, including cancer (Ascoli,¹ Grünbaum,¹³ and others), was capable of agglutinating the corpuscles of normal individuals, while the corpuscles of another individual with the same disease were immune to this action. This is the phenomenon of so-called isoagglutination. The Italian observers claimed that this biological reaction was a safe diagnostic criterion of malaria.²³ The careful researches of Landsteiner,¹⁸ ¹⁹ since verified by Donath,⁶ Gay,³⁵ and many others, threw grave doubts on all these results by proving definitely that there was a certain series of formulæ, three in number, to which the agglutinating powers of all human sera — normal and pathological — could be shown to conform, and that there was nothing in any manner characteristic of the reaction in any form of disease.

Partly contemporaneous with this work on agglutination, but mostly later, came a number of researches which dealt with the lytic power of human serum on the red cells of the blood. This work, as bearing more directly on the subject matter of this paper, demands special attention. Certain observers studied the action of serum in various diseases on the corpuscles of alien species. One of the most noteworthy of these researches, unfortunately cut short by his early death, came from J. M. Polk,²⁵ who succeeded in clearing up a great many of the baffling intricacies of the technic. Kelling¹⁵ tested the serum of human cancer cases upon the corpuscles of sheep, cows, and chickens, and found that in forty-three per cent of the cases its hemolytic power greatly exceeded that of normal human serum. His theory of the specificity of this reaction in cases of cancer was attacked by v. Dungern,⁸ and recently by Fischel.¹¹ The latter author has, within the current year, tested the sera in a variety of human diseases upon the red cells of chickens. He finds that in about fifty per cent of the cases of malignant tumors the blood has a markedly increased hemolytic power as compared with the

normal. On the other hand, he finds the same reaction to be present in some cases of diabetes, pernicious anemia, chronic endocarditis, and tuberculosis. It is, therefore, not specific in tumors. It is an interesting fact, as is pointed out by Fischel, that his observations are in perfect accord with those which I reported to the American Association for Cancer Research in 1907,³⁸ although in his experiments the human serum was tested against alien corpuscles, and in mine against human red cells.

Recently the tendency has been rather to study the lytic effect of human serum on the corpuscles of other human individuals. Ascoli,¹ in 1901, published the results of such a study in one hundred and fourteen human individuals, of whom seventeen were normal, ninety-seven diseased. He found that the normal sera were, as a rule, not hemolytic at all, or at most only slightly so.

He found no hemolysis in five cases of chlorosis, two cases of anchylostomiasis, one of liver abscess, three of acute rheumatism, three of exudative pleurisy, two cases of plumbism, one of acute Bright's, two of chronic Bright's, and a large number of cases of bronchitis and of gastritis. On the other hand, he found that hemolysis was pronounced in two cases of gastric cancer, one of pneumococemia, and in a large number of cases of tuberculosis, and of pneumonia. Kreibich¹⁷ (1902) found that the serum had no hemolytic power in six cases of pemphigus, ten of erysipelas, five of lues, three burns, one purpura, and one staphylococemia. Eisenberg¹⁰ found that of fifteen cases of typhoid, ten had hemolytic sera; of eight cases of scarlatina, seven hemolyzed; and of eight syphilitic subjects, five hemolyzed.

The net result of these and of some other less extensive researches was to demonstrate that human serum in various diseases is occasionally hemolytic towards the corpuscles of other human individuals. The mechanism of this hemolytic action of serum was beautifully illustrated by the researches of Landsteiner and Donath⁷ on cases of paroxysmal hemoglobinuria. At the same time, it was clear that this hemolytic phenomenon, interesting and striking as it was, could not be considered of diagnostic value, inasmuch as it was present in so many and such varied conditions of disease.

In 1907 I described a new method of studying hemolysis in disease, based not only on the reaction of the serum, but also on the degree of resistance of the corpuscles to that serum. It is of importance for the understanding of that method and of the results which it has yielded, not only in my hands, but in the hands of other investigators, to describe in some detail the work which has led up to it. The material on which the earlier work was based consisted of a large number of dogs affected with the type of tumor known as infectious lymphosarcoma, which were put at my disposal by S. P. Beebe. The character of these tumors and their mode of growth have been fully described by Beebe and Ewing.² The first step in the study was to investigate the action of these tumors, when removed from the body and extracted in salt solution, on the red cells of the dog. The same method had been previously employed by a number of observers in the study of human tumors, but necessarily under far less satisfactory conditions. As a result of a large number of experiments,^{29, 30} it became clear that the tumors of dogs, as well as most of their normal organs, when freshly extracted, possess the power of destroying to a very slight extent the corpuscles of other dogs, and that this power can be slightly enhanced by adding to the tissue extract an extract of red blood cells. The latter phenomenon has been described by Sachs²⁶ in the case of hemolysis by cobra venom, the red cell constituent of the reaction, which in my paper was called "red-blood-cell-derivative," or R. B. C. D., being designated by him as endo-complement. Far more striking, however, was the effect of necrosed or broken down portions of these same tumors upon the red cells; under these conditions the red cells were rapidly deprived of their coloring matter and even completely destroyed. On the theory that this result might be due to the presence of autolytic products in these broken down tumors, organs of the dog were autolyzed antiseptically outside of the body, and a number of intra vitam necroses were produced by tying off the vessels of the kidney. It was found that these autolyzed tissues produced hemolysis in exactly the same

fashion as did the necrotic tumors. This work has recently been repeated and verified by Fukuhara.¹² It is therefore quite evident that the broken down tumors contain a material very poisonous to the red cells of the blood. The clinical aspect of this fact is revealed in the observation that cachexia, as Beebe has found in his dogs, is associated not with the progressive growth of these tumors, but with the process of necrosis and softening. It was the theoretical anticipation of this fact which led F. Müller,²⁴ in 1889, to attribute the cachexia of cancer to a "toxogenous disintegration of protoplasm, independent of nutrition," in other words, to a specific toxic effect of the broken-down tumors.

The next step was to determine whether this supposed toxic material, or any characteristic effect of its activity, could be traced in the circulating blood of dogs affected with the growth.^{31, 32} Blood was therefore taken from a large number of dogs, including some with tumors and some without, and the effect of the sera upon corpuscles was determined in a series of experiments, the results of which were published during the past year. These experiments showed conclusively that a considerable proportion of dogs with tumors, especially when in the stage of necrosis, possessed a serum which is hemolytic in some degree for all dog corpuscles. Normal dog serum is very rarely hemolytic, and, if so, generally in a very slight degree. In the second place, the corpuscles of dogs with tumors are far more resistant to this hemolytic activity of the serum of tumor dogs than are the corpuscles of normal animals. In other words, the serum of tumor dogs frequently contains an "isolsin," and their corpuscles are relatively immune to its action. The phenomenon is precisely comparable to the existence of isoagglutinins and the immunity of the corpuscles to these agglutinins in certain groups of human individuals, as demonstrated by Landsteiner.

The existence of such a toxin in the circulating blood of dogs affected with these tumors has gained additional support from the observation of Wade³⁴ that the growth of the tumor is associated with the production of an interstitial

nephritis. The supposed "toxin can be isolated from it (the tumor) by filtration, and produces interstitial nephritis" of the same character experimentally.

The existence of a double reaction of the type just described is evidently a very different matter from the simple hemolytic reaction described by Ascoli or Eisenberg. It is conceivably capable of assuming diagnostic importance, inasmuch as there appears in dogs, at least, to be something specific in the relative resistance of the corpuscles to the hemolytic activity of the serum. This consideration led, upon the completion of the work on dogs, to the study of the blood of human subjects suffering from new growths. (These results have already been reported in brief at the first and second meetings of the American Association for Cancer Research.^{31,33}) I have had at disposal the material of two large general hospitals, the German Hospital and the Sydenham Hospital, and a few cases in Bellevue, kindly furnished by Dr. Coleman. The work was done on a selected set of eighty-two cases, in all of which the diagnosis was fairly certain, being confirmed by microscopic examination of autopsy or operative material in the group of tumors. There were thirty-one cases of malignant tumors, of which fifteen were in an early stage and sixteen were in an advanced condition, using the terms "early" and "late" in a broad clinical sense. There were three cases of benign new growth. There were forty-two cases of other diseases. Six of the cases were apparently normal. The method was that previously described. From ten to twenty cubic centimeters of blood was aspirated from the median basilic vein; part of this blood was allowed to clot and the serum was poured off, while the rest was defibrinated, and the washed corpuscles made up into a two per cent emulsion in normal salt solution. In every experiment, the serum was tested both against its own corpuscles and against the corpuscles of another individual described as the "control;" after some experimentation it was decided to select as a "control" either a normal individual or one with a different type of disease. The results obtained in all these experiments are given in the accompanying table (Table I.).

TABLE I.
The hemolytic reactions in eighty-two human cases.

Diagnosis.	Pathological Examination.	Remarks.	Hemolysis of	
			Own Corpuscles.	Alien Corpuscles
I. Malignant tumors :				
Tumor of lip.....	Carcinoma.	Early.	—	—
Tumor of lip.....	"	"	—	+
Tumor of tongue	"	"	+	+
Tumor of tonsil.....	"	Late.	—	—
Tumor of larynx.....	"	Early.	—	+
Tumor of esophagus	"	Late.	+	—
Tumor of stomach.....	"	Early.	+	—
Tumor of stomach.....	"	"	+	+
Tumor of stomach.....	"	"	—	+
Tumor of stomach (4 cases) ..	"	Late.	—	+
Tumor of colon	"	"	—	+
Tumor of sigmoid	"	"	—	—
Tumor of rectum	"	Early.	—	—
Tumor of prostate (2 cases) ..	"	"	—	—
Tumor of penis.	"	"	—	—
Tumor of breast	"	"	—	+
Tumor of breast (3 cases).....	"	Late.	—	+
Tumor of breast	"	"	+	+
Tumor of cervix	"	Early.	—	—
Tumor of vaginal scar.....	"	"	—	—
Tumor of kidney	"	Late.	+	+
Tumor of face	Epithelioma.	"	—	—
Tumor of scalp	"	Early.	—	+
II. Benign tumors :				
Tumor of uterus	Fibroid.	—	+
Tumor of uterus	"	—	—
Tumor of breast	Adenoma.	+	—
III. Other diseases :				
Plumbism (3 cases).....	—	—
Pneumonia.....	—	—
Pneumonia	—	+
Pernicious anemia (2 cases)	—	—
Leukemia.....	—	—

TABLE I. — *Continued.*

Diagnosis.	Pathological Examination.	Remarks.	Hemolysis of	
			Own Corpuscles.	Alien Corpuscles.
Hodgkin's			+	—
Malaria (2 cases)			—	—
Tuberculosis of lungs (2 cases)			—	+
Tuberculosis of joints			—	+
Tuberculosis of cervical glands			—	—
Syphilis			—	+
Syphilis			—	—
Typhoid (2 cases)			—	—
Acute rheumatism			—	—
Chronic rheumatism			—	—
Gout			—	+
Cerebral embolism			—	—
Chronic endocarditis (2 cases)			—	—
Abscess of liver		Amebic.	—	—
Obstructive jaundice			—	—
Cirrhosis hepatis		Ascites, no jaundice.	—	—
Pancreatitis			—	—
Chronic gastritis			—	—
Chronic colitis			—	—
Chronic mastitis			—	+
Appendicitis		Abscess.	—	—
Hernia			—	—
Neurasthenia (2 cases)			—	—
Senility			+	—
IV. Normal (6 cases)			—	—

It is apparent that the results may be grouped under four headings, as follows:

1. Serum fails to hemolyze its own corpuscles, but hemolyzes alien corpuscles.
2. Serum hemolyzes both its own and alien corpuscles.
3. Serum hemolyzes its own, but not alien corpuscles.
4. Serum hemolyzes neither its own nor alien corpuscles.

The results have therefore been summarized according to these four categories, in Table II., in which the first group is indicated as — +, the second as ++, the third as + —, and the fourth as — —.

TABLE II.
(Summary of Cases.)

	I.	II.	III.	IV.
	— +	++	+ —	— —
1. Early malignant tumors.....	6	2	1	6
2. Late malignant tumors.....	9	2	2	3
3. Benign tumors.....	1	0	1	1
4. Other diseases.....	11	0	2	29
5. Normal cases.....	0	0	0	6

Group I. (— +) conforms to the type of reaction described in the lymphosarcomata of dogs, and may be called a "positive" reaction. Of the early malignant tumors, six (forty per cent) conformed to this type; of the late malignant tumors, nine (fifty-six per cent) conformed to it; of the benign tumors, one (thirty-three per cent), and of the other diseases, eleven (twenty-six per cent) showed this type of reaction. It is evident, therefore, that the reaction is not pathognomonic of malignant tumors, early or late, that it occurs in a considerable proportion of other diseases, and that a large proportion of tumors present an altogether different type of reaction. On the other hand, it must be admitted that a much larger percentage of malignant new growths present this type of reaction than do cases of other disease. The results of the experiment are, on the whole, far less characteristic than in case of the tumors of dogs, and do not lend themselves at present to diagnostic application. There are a number of obvious explanations for these differences between dogs with lymphosarcoma and human beings with malignant new growths. In the first place the former

present a single and sharply demarcated type of growth of which all the cells are of one stock and belong to one family. In human beings, however, the group of cancers comprises a collection of growths which differ very markedly, not only in their morphology, but also in their biological characters. A thyroid cancer, as is shown by the well-known case of v. Eiselberg, may save the body from myxedema; an epithelioma of the face, even though far advanced and inoperable, often produces hardly any constitutional effects; a tumor of the stomach, even though of slight extent, may destroy its subject (without producing pyloric stenosis). Striking differences such as these between the various types of new growth grouped as "cancer" might easily be multiplied, showing that the action upon the organism as a whole may, on the one hand, be intensely destructive, or, on the other, even physiologically compensatory in some respects, with every gradation between these two extremes of action. It is therefore unreasonable to expect that such different conditions should all produce a uniform alteration of the serum. In spite of this difficulty I believe that some importance must be attached to the fact that the sera of individuals with tumors present a far larger proportion of "positive" (— +) reactions than do other individuals, whether normal or diseased. It seems very possible, therefore, that something of value may eventually come of the use of this method in human disease. But it will have to come with a refinement of method which will correspond to the complexity of the factors involved.

The importance of the method in its application to human disease has been recently given considerable prominence by Crile.⁵ He studied hemolysis and corpuscular resistance, according to the method described by me, in cases of human cancer, and with slight variations he exactly confirms my conclusions in the study of the blood of dogs. He states that all early cases of malignant new growth have a hemolytic serum, and that the corpuscles of such cases are immune to the destructive action of their own serum, or of the serum of other cancer cases. As the tumors progress, he says that

the hemolytic power of the serum disappears, and with it the resistance of the corpuscles. This reaction he finds to be characteristic of cancer. The only apparent exception is found in cases of tuberculosis. He finds that the reaction is so delicate as to betray the very earliest beginnings of malignant new growths as, for example, in cases in which the "cancerous transformation" of a luetic scar or the "sarcomatous transformation" of a fibroid of the uterus were evident only on microscopic examination. It is evident that a method as delicate and at the same time as unerring as this would be of great use in practice. My own observations do not permit of such a sweeping generalization, and I believe that it will hardly stand the test of further investigation. The method yields results of such a character that great caution and reserve must be exercised in their application to human diagnosis.

The theoretical aspect of this type of hemolysis is of great interest, and has not hitherto been presented. It is, on first thought, somewhat difficult to conceive that a substance destructive to the life of red blood cells—a hemolysin—should circulate in the blood of the living animal. Evidently, if such a substance does exist, the cells of the animal in which it occurs must be immune to its action. Otherwise, there would be progressive destruction of the red cells and death. Now the observations of many careful workers show that just these conditions do actually exist. Landsteiner and many others have demonstrated the presence of isoagglutinins in human blood, and the existence of a specific immunity to their action on the part of the cells of the individual in which they occur. Yet these same cells may be very susceptible to agglutinins present in the blood of other individuals of the same species. Do the same laws hold of hemolysins? Until recently it was not known that hemolysins for the red cells of the same species could normally occur. Ehrlich⁹ had indeed shown that such hemolysins (iso-hemolysins) could be artificially induced in goats by injecting the red cells of other goats. In these injected animals, however, the serum never became hemolytic

for their own cells. Ehrlich called this phenomenon the "horror autotoxicus," which is simply another way of stating that their own red cells had become immune to their own hemolysin. In 1906 Theobald Smith²⁷ showed that isolysins normally occurred in a considerable number of horses, which were capable of destroying fifteen per cent of the corpuscles of other horses. In 1907 I showed the same to be true in some dogs not affected with tumors. There is, therefore, no doubt that animals may have in their blood hemolysins for the red cells of their own species, but not toxic for their own cells. The question now arises, can the red cells of an animal become immune to the action of a hemolysin introduced into the blood from without during adult life? For example, could the red cells become immune to a toxin circulating in the blood and derived from a disintegrating new growth? There is ample evidence to show that red cells may change in their resistance to anisotonic salt solution during the course of disease.^{4, 21} This, however, is not a specific response. There is, as far as I know, only one condition in which it has been abundantly shown by independent observers that there may be such a specific immune response on the part of the red cells. If eel serum be injected a number of times into rabbits,^{3, 16, 28} their red cells become relatively immune to its destructive action in the test-tube. The mechanism of this response need not be very complex. New red cells are being constantly manufactured, and it is only necessary to imagine that those less resistant to the poison die off, leaving only those more resistant. There is, therefore, no inherent difficulty in the conception of an isolysin due to disease and a corpuscular immunity thereto. On the other hand, we are very far from having proved that this is the actual explanation of the hemolytic reactions of the blood in certain forms of disease, specifically in the lymphosarcoma of dogs. It is conceivable that the phenomenon is due to an entirely different set of factors. This is a problem which only much labor and care can hope to solve.

BIBLIOGRAPHY.

1. Ascoli. Münch. Med. Wochenschr., 1901, 1239.
2. Beebe and Ewing. J. Med. Research, 1906, 209.
3. Camus and Gley. Arch. de pharmacodyn. int., 1889, 247.
4. Chanel. Thèse de Lyon, 1880.
5. Crile. J. A. M. A., 1908, 1, 1883; li, 158.
6. Donath. Wien. Klin. Wochenschr., 1900, 497.
7. Donath and Landsteiner. Ztbl. f. Bakt., Vol. 45.
8. Dungern. Ztschr. f. Krebsforschung, 1907.
9. Ehrlich and Morgenroth. Berl. Klin. Wochenschr., 1900, No. 21.
10. Eisenberg. Cit. by Kreibich, l.c.
11. Fischel. Berl. Klin. Wochenschr., 1908, 882.
12. Fukuhara. Ztschr. f. Exp. Path. u. Ther., 1907, 658.
13. Grünbaum. Brit. Med. J., 1900.
14. Halban. Wien. Klin. Wochenschr., 1900.
15. Kelling. Berl. Klin. Wochenschr., 1907, 1355.
16. Kossel. Berl. Klin. Wochenschr., 1898, 152.
17. Kreibich. Wien. Klin. Wochenschr., 1902, 699.
18. Landsteiner. Centrbl. f. Bakt., 1900.
19. Landsteiner. Wiener Klin. Wochenschr., 1901.
20. Landsteiner and Sturli. Wiener Klin. Wochenschr., 1901.
21. Lang. Ztschr. f. Klin. Med., 1902.
22. Limbeck. Prager Med. Wochenschr., 1890.
23. Lo Monaco and Panichi. Sitzungsber. d. Accad. dei Lincei, 1900, Dec. 16.
24. Müller. Zeitschr. f. Klin. Med., 1889, 496.
25. Polk. J. Med. Research, 1904.
26. Sachs. Berl. Klin. Wochenschr., 1902, No. 38.
27. Smith and Brown. J. Med. Research, 1906, 425.
28. Tshistovitch. Ann. de l'Inst. Pasteur, 1899, 406.
29. Weil. Proc. Soc. Exp. Biol. and Med., 1907, 25.
30. Weil. J. Med. Research, 1907, 287.
31. Weil. J. A. M. A., 1908, 1, 64.
32. Weil. Arch. Int. Med., 1908, No. 1.
33. Weil. J. A. M. A., 1908, li, 158.
34. Wade. J. of Path. and Bact., 1908, 384.
35. Gay. Proc. Soc. Exp. Biol. and Med., 1907, 14.

A STUDY OF NATURAL AND ACQUIRED IMMUNITY OF GUINEA-PIGS TO GONOCOCCUS.*

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It is a matter of common knowledge that man after an attack of gonorrhea does not acquire the slightest immunity to a subsequent infection by the micrococcus of Neisser. In fact, the resistance of his system to invasion by the gonococcus is decreased by each recurring attack. The majority of investigators have concluded that a specific immunity in animals to the gonococcus is unattainable; a result due in part to the fact that fluid cultures, in which gonotoxin has accumulated, have been used as the immunizing agent. It is my purpose in this paper to demonstrate that such a generalization is unwarranted and also to describe the mechanism of the specific immunity to this microörganism, which may readily be induced in guinea-pigs. Before passing to this subject, it is desirable to consider the effect of the injections of living gonococci and also its diffused toxin on normal animals.

I. Toxin in filtrates of gonococcus cultures. The nature of gonotoxin.—If gonococcus is cultivated for two or three weeks in a suitable fluid medium, such as ascitic broth, the filtrate will be found to be more or less toxic for small laboratory animals. With the exception of one investigator, it is generally agreed that this toxic substance is derived entirely from the dead and disintegrated bodies of the gonococci and is not a physiological product of the living organism. Nicolaysen,¹ Wassermann,² and Scholtz³ were the first to establish this point. Christmas,⁴ however, by the use of a special culture medium, claims that the gonococcus may produce, by a biological process, an extracellular

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poison, which, when injected into the brain of a guinea-pig, is extremely toxic.

With the idea of testing the validity of Christmas' theory, I have repeated some of his experiments. The formula which he considers essential is an infusion of veal, concentrated to one-fourth its original bulk, to which three parts of ascitic fluid have been added, and neutralization effected with lactic acid. Peptone and salt, he claims, have an inhibitory influence on toxin production. The toxic filtrate was obtained by passing the culture through sterile talc. A comparison of the effect of intraperitoneal injection into guinea-pigs of filtrates of cultures grown in this medium with others, to which peptone, salt, and ascitic fluid have been added in varying proportions, leads one to the conclusion that no enhanced virulence is secured by the formula of Christmas. Intracerebral injections were not made, because the severity of the operation adds a complicating factor. Vannod⁵ and Cantoni⁶ have reported a similar conclusion as regards Christmas' medium. It has been my experience that the filtrate of the formula in which there is the greatest growth and hence the most active disintegration of cocci contains the maximum amount of toxin. Peptone increases the growth and hence the toxicity of the culture. Nor is ascitic fluid essential, for the filtrates of two cultures, one grown in ascitic broth (Thalmann's⁷ broth, two parts; ascitic fluid, one part) and the other in Thalmann's broth without ascitic fluid, were found at the end of twenty days, which is, according to Christmas⁴ and Vannod,⁵ the time of the maximum production of toxin, to be equally poisonous on injection into the peritoneal cavity of guinea-pigs. Unless, however, the conditions are very favorable it is not possible to obtain an equal growth in serum broth and broth without serum. Such a deficiency would account for Wildbolz's conclusion that the cultures in the former are the more toxic. My results do not exclude the possibility that a culture may lose considerably in virulence if cultivated for some time in media devoid of serum, but they do lead one to believe that

there is no vital interaction between the culture fluid and the gonococcus in the production of toxin.

In connection with some experiments on the fixation of complement with gonococcus, carried out in collaboration with Dr. Teague,⁸ we found that neither filtrates of old ascitic broth cultures of gonococcus nor extracts obtained with distilled water and also seventeen per cent sodic chloride solution caused hemolysis of washed sheep blood corpuscles. It seems probable, then, that in these experiments with guinea-pigs a hemolysin played no part in the toxic action.

Immunization to gonotoxin. — In regard to the possibility of immunization to gonotoxin and the production of an antitoxin there is a similar diversity of opinion. Christmas,⁴ using his special technic, claims to have produced an immunity in guinea-pigs which enabled them to withstand one hundred minimal fatal doses injected intracerebrally. The serum of an immunized goat was also described as possessing antitoxic properties. On the other hand, Wassermann,² as also Jundel⁹ and Vannod⁵ have found that no immunity, other than a slight adaptation and resistance to gonotoxin, could be induced in small laboratory animals, nor is antitoxin production possible.

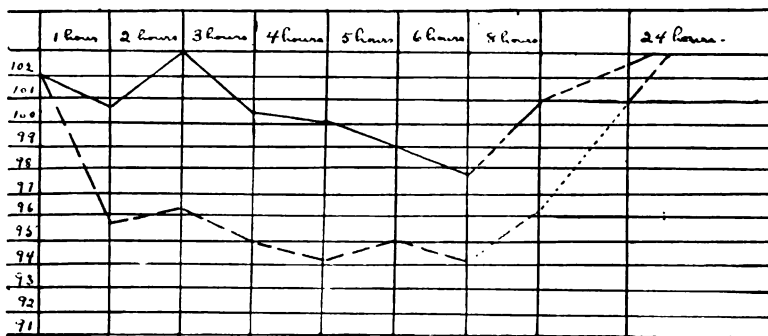
To verify one or the other of these conclusions I have experimented on the immunization of guinea-pigs against toxin filtrates injected intraperitoneally and produced both according to the method of Christmas, and also by various modifications of ascitic broth. The inoculations in a little less than fatal doses were given at intervals of a week or more. In no instance, however, was there evidence of the production of active immunity. Although, after the third or fourth injection, there might be some indication of slight tolerance, on further treatments this frequently gave way to a sudden hypersensitivity. Other pigs became marasmic and succumbed after progressive emaciation, as has been described by Wassermann,² Jundel,⁹ and Moltschanoff.¹⁰ Occasionally, partial paralysis preceded death, appearing first in the hind limbs and then in the fore limbs. Moltschanoff¹⁰

has shown that this paresis is accompanied by degenerative changes in the nerve cells of the anterior horns of the spinal cord.

Evidence suggestive of anaphylactic phenomena. — In a number of instances, the first injection of toxin seemed to sensitize the animals in such a way that they were much more distressed by a second injection in the same dose given fifteen to twenty-three days later. This hypersensitivity is shown graphically by the more rapid and extensive drop in temperature after the second inoculation (Temperature chart No. 1).

TEMPERATURE CHART NO. 1.

The unbroken line (—) indicates the temperature changes in a guinea-pig after the first intraperitoneal injection of a filtered gonotoxin. The broken line (— — —) is the curve of the same animal receiving the same dose of toxin twenty-three days later.



This increased susceptibility was more strikingly evident in some animals after several injections had been given. Six pigs died suddenly in two to ten hours after the inoculation of less than an ordinarily fatal dose, in some instances without any drop in temperature. In each case an interval of eight to fifteen days had occurred since the dose immediately preceding. A rapid onset of convulsions and dyspnea before death was also suggestive of anaphylaxis.

II. Experiments on the injection of emulsions of living gonococci into normal guinea-pigs. Variations in virulence.

—In experimenting with twelve different strains of gonococcus isolated from various sources, great difference in toxicity were encountered. But with the most virulent strains it was necessary to use a relatively large amount of culture to produce death. A constantly toxic culture, called A in previous articles, has been employed in the majority of the experiments. This strain has lost its virulence very slowly during the eighteen months in which tests have been made. At the beginning of this period the twenty-four-hour growth from three-fourths of a square inch of an ascitic agar slant would sometimes prove fatal to a two-hundred-gram guinea-pig and one and one-half square inches certainly so. As Wollstein¹¹ in similar experiments found it necessary to use half of the growth from a "Blake" bottle to bring about a fatal issue, this strain was evidently far less toxic than A. At the end of twelve months one and one-half square inches of culture A was sometimes fatal and three square inches certainly so. Finally at the present time the certainly fatal dose is about four square inches for a two-hundred-gram pig.

Dosage. — At all times the dosage has been graduated according to the weight of the animal, although large pigs are relatively less susceptible than small. For a standard emulsion three square inches of a luxuriant eighteen to twenty-four-hour ascitic agar culture have been suspended in four cubic centimeters of normal saline solution and this emulsion injected in a dosage of one cubic centimeter to each hundred grams. These inoculations were always made intraperitoneally.

Physical phenomena following injections. — In the case of pigs which die within nine to sixteen hours after injection the temperature drops rapidly and steadily for eight to ten degrees (F.) in three hours. If death is delayed until twenty to twenty-four hours the fall in temperature is slower, sometimes preceded by a slight rise during the first two hours. After the injection of a little less than a lethal dose the temperature rises during the first hour, falls rapidly at four hours and finally rises again to the normal in nine to ten hours. The peritoneal cavity begins to fill with a clear exudate in from one to three hours and soon becomes much distended and very tender, with tense abdominal muscles. In fatal instances, convulsions occur a few hours before death. There may be paresis of the hind limbs and a prolapsus ani. Similar phenomena have been noted by Flexner¹² in pigs inoculated with a fatal dose of the meningococcus. In surviving animals there is a resorption of the peritoneal fluid, simultaneous

with the rise in temperature. As Nicolaysen¹ observed, death does not occur later than thirty-six hours, unless a mixed infection has set in.

Autopsies. — At autopsy in guinea-pigs dying in ten to twenty hours the peritoneal cavity is found to be filled with a yellowish fluid, clear or slightly bloody. The omentum is rolled up on the stomach and much inflamed, as is also the mesentery and the serosa of the peritoneum. As Vannod⁵ noted, the suprarenal capsules are enlarged and intensely hyperemic. In later fatal cases the conditions are the same except that the fluid has generally become scanty, viscid and clouded. Deposits of pus lie on the liver and abdominal walls. The gall-bladder is sometimes enormously distended in animals which recover later and especially in those immunized with toxin; a condition described by Detweiler¹³ and also by Tracy¹⁴ as occurring in guinea-pigs killed by toxins of *B. prodigiosus*.

Tabulation of normal animals. — In the following table are given data in regard to guinea-pigs which were killed or died in five minutes to seventy-two hours after inoculation. At the periods up to ten hours, death was induced by chloroform, each animal having received intraperitoneally the same dose, viz., four cubic centimeters of the standard emulsion of culture A. Cultures from the peritoneal fluid and heart blood were taken by using the same platinum loop, holding approximately 1/140th of a cubic centimeter and smearing one loopful over the surface of a large ascitic agar slant. After forty-eight hours' incubation the colonies were counted, when possible.

TABLE I.
Series of normal guinea-pigs inoculated intraperitoneally with a standard dosage of gonococcus A.

Pig.	Time of Death or Chloro- forming.	Culture, Peritoneal Fluid.	Culture, Heart Blood.	Smear of Peritoneal Fluid.	Omentum.	Remarks.
180.	5 minutes. Dead. Chloroforming begun in 3 minutes.	48	Gonococci not clumped. A few megakaryocytes and small lympho- cytes. No phagocytosis.	Deposit of fibrin containing great quantities of gonococci and leuco- cytes. No phagocytosis.	Suprarenal yellow.
197.	4 minutes. Dead. Chloroforming begun in 1 minute.	50	Gonococci abundant, not clumped, stain well. Few leucocytes. No phagocytosis.	Fibrin just beginning to be deposited. Bunches of "hyaline" cells with gonococci stuck to them. Many "trailers." No phagocytosis.	Omentum not rolled up. Suprarenal yellow.
198.	30 minutes.	350	Fig 198. Lymphocytes and "hyaline" cells very abundant. Some contain a few gonococci. No megakaryocytes or microcytes. Gonococci abund- ant, scattered, stain well.	Slight deposits of fibrin and few free gonococci. "Trailers" abundant, have taken up a moderate number of gonococci, which appear normal.	Omentum slightly rolled up. Suprarenal yellow.
181.	35 minutes.	160			
183.	1 hour.	140	Gonococci abundant, stain well, no agglutination. No leucocytes.	Moderate deposits of fibrin filled with gonococci.	Omentum rolled up, hyperemic. Supra- renal yellow.
199.	1½ hours.	500	Gonococci very abundant, scattered, stain well. Very few leucocytes.	Extensive fibrin deposits containing many cocci, which stain well. Great many "trailers," most have taken up a few cocci. Same true of round macrophages.	Abdomen distended. Suprarenal slightly reddish.

TABLE I. — *Continued.*

Pig.	Time of Death or Chloro- forming.	Culture, Peritoneal Fluid.	Culture, Heart Blood.	Smear of Peritoneal Fluid.	Omentum.	Remarks.
184.	2 hours.	500	Fig 191. Gonococci stain very well, scattered. No leucocytes.	Moderate deposit of fibrin. Macro- phages have ingested many more gonococci than the micrococci. Gonococci stain well.	Omentum rolled up. Su- prarenal reddish.
191.	2 hours.	∞ Luxuriant.	350			
192.	4 hours.	∞ Luxuriant.	20	Fig 192. Gonococci not as numerous as in 2 hours. Stain well and scat- tered. Very few leucocytes.	Fibrin scanty. Great quantities of macrophages and micrococci. Macrophages stuffed with gonococci which stain well. Some also in micrococci.	A great deal of fluid in the peritoneal cavity. Suprarenal reddish.
185.	4 hours.	∞ Not luxuriant.	21			
193.	6 hours.	∞ Luxuriant.	2	Fig 194. Gonococci numerous, stain well, scattered. No leucocytes.	Moderate deposit of fibrin enclosing leucocytes and gonococci, the latter stain well. Very few micrococci. Great many "trailers" — not phago- cytic.	T. 98 1/5. Moderate amount of peritoneal fluid. Suprarenal slightly hyperemic.
194.	6 hours.	∞ Less growth than in 193.	300			
129.	6½ hours.	∞ Luxuriant.	4	Moderate number of scattered gono- cocci, which stain fairly. No leuco- cytes.	Extensive deposit of fibrin containing great numbers of micrococci. Many scattered gonococci.	T. 91. Nearly dead when killed. Peritoneum injected. Suprarenal strongly hyperemic.

195.	10 hours.	2,000 + —	1	Pig 105. Microxycytes have emigrated in considerable numbers. A few macrophages and megxycytes. Few gonococci, found here and there in leucocytes.	Great many microxycytes about fibrin. Many macrophages and microxycytes filled with gonococci, which stain intensely.	T. 93. 4/5. Peritoneum quite inflamed. A good deal of exudate. Suprarenal strongly hyperemic.
196.	10 hours.	10,000 + —	2			
148.*	14 hours. Dead.	∞ Luxuriant.	30	Pig 142. Gonococci very numerous, stain well, may have multiplied. Moderate number of microxycytes, not as many as in pigs recovering. Microxycytes have ingested from 5 to 15 cocci.	Great many free gonococci, some stain well, others pale and swollen. Few microxycytes. These and macrophages have taken up great quantities cocci, which stain well.	Peritoneal fluid very abundant. Hemorrhagic spot on stomach. Suprarenal strongly hyperemic.
142.*	16 hours. Dead.	∞ Luxuriant.	50			
153.*	16 hours. Dead.	∞ Luxuriant.	80			
156.*	18 hours. Dead.	∞ Luxuriant.	15	Pig 156. Many scattered gonococci, which stain fairly. Do not seem to have decreased in number. Very few leucocytes, not more than sometimes at two hours.	Peritoneal fluid abundant and clear. Suprarenal hyperemic.
90.	18 hours. Dead.	∞ Good.	150			
143.*	20 hours. Killed.	∞ Fair.	1	Pig 89. No gonococci. Very few leucocytes. A few microxycytes, vacuolated and agglutinated, contain a small number of cocci.	A good deal of fibrin, but very few microxycytes. Very few gonococci to be seen.	Peritoneal fluid very abundant and slightly clouded.
89.	22½ hours. Died.	60	1			
131.	24 hours. Died.	200	1	A considerable number of microxycytes, mostly actively phagocytic. Macrophages jammed with cocci and vacuolated. Megxycytes to some extent phagocytic.	A good microxycytic reaction. These leucocytes actively phagocytic. Macrophages vacuolated and have taken up microxycytes.	Peritoneal fluid quite abundant, thick and yellowish.

TABLE I. — *Continued.*

Fig.	Time of Death or Chloro- forming.	Culture, Peritoneal Fluid.	Culture, Heart Blood.	Smear of Peritoneal Fluid.	Omentum.	Remarks.
87.	25 hours. Killed.	100	0	Fig 87. No free gonococci. Great quantities of microxyocytes. Macrophages have not ingested microxyocytes.	Not many microxyocytes. A large number of gonococci, staining well, scattered over the surface.	T. 96 1/5. Still sick. Peritoneal fluid quite abundant, thick, viscid.
88.	25 hours. Killed.	0	0			
94.	25 hours. Killed.	5	0			
82.	26 hours. Died.	Slight growth.	0	Fig 82. Microxyocytes and macrophages very numerous. A good many cocci in the macrophages.	Very few microxyocytes. No gonococci.	Fig paralyzed. Peritoneal fluid abundant, thin.
220.	28 1/2 hours. Killed.	3	0			
53.	48 hours. Killed.	0	0	Macrophages have taken up many microxyocytes. A few extra cellular cocci still present, but probably dead.	Peritoneal fluid scanty thick, slightly bloody.
57.	72 hours. Killed.	0	—	Mostly macrophages and endothelial cells. The macrophages have taken up and digested all the microxyocytes.	No peritoneal fluid. No inflammation.

* Cultures inoculated, which had been increased in virulence, to a varying degree, by passages.

Cultures. — The peritoneal fluid gave a luxuriant culture up to six hours. From this time to ten hours a marked decrease in the living gonococci occurred and the animals would, in all probability, have recovered. The animals, however, which died within eighteen hours still held enormous numbers of gonococci, either because practically none had been destroyed or less probably on account of a multiplication of the cocci in the animal after a primary destruction. As is indicated in the table all except one of the pigs dying in this period had received a culture somewhat increased in virulence by passages through animals. Scholtz³ also found that, from guinea-pigs dying within twenty hours after inoculation with gonococci, they may almost invariably be recovered in culture. After twenty-four hours, whatever the outcome, there are few or no living gonococci in the peritoneal fluid. As might be expected gonococci may readily be recovered from the heart blood after intraperitoneal inoculation. Nicolaysen,¹ Scholtz,³ Jundel,⁹ and Wildbolz¹⁵ obtained them in culture with varying degree of frequency. Bruckner¹⁶ found them in smears of the heart blood and by culture in the peripheral circulation during the first hours. In this series of pigs they were recovered more or less abundantly from the heart blood up to twenty-four hours. Even in five minutes after inoculation there were fifty gonococci in a loop, indicating that there has occurred the same rapid rush through the diaphragm which Buxton and Torrey¹⁷ have found to be true for *B. typhosus* and other bacteria. The maximum number of cocci was found in two hours, as many as five hundred in a loop or seventy thousand in a cubic centimeter. As most pigs recover after this dose, it is a striking demonstration of their natural resistance to the gonococcus. At four hours a marked decrease has occurred as there were only twenty cocci to a loop. The bactericidal action continues in animals which are on the road to recovery until in ten hours the blood is practically sterile. Yet, as the table shows, in the blood of animals dying up to twenty hours a large number of living cocci are still present. After twenty-five hours both in dying and surviving pigs a complete

destruction of the cocci has almost invariably taken place; hence there is at times a close approximation to "sterile death."

Peritoneal fluid. — Examinations of the peritoneal exudates are often instructive as an indication of the course of the struggle between the protective agencies of the animals and the bacteria injected. The smears were stained with eosinate of methylene blue. During the first hour the gonococci are abundant, well scattered and stain intensely, while the leucocytes are few in number and of the usual character (Dudgeon and Ross¹⁸). The prolonged leucopenic stage, which is well marked in two hours, extends until about seven hours, when the polynuclear leucocytes with small granules (microxyctes) begin to appear in numbers. If the animal is destined to recover, these cells continue to emigrate in large quantities until in twenty-five hours they far outnumber the macrophages. As is well known, the macrophages soon gain the upper hand and in seventy-two hours have completely destroyed the microxyctes. Since in the case of pigs recovering in less than twenty hours the gonococci injected are mostly destroyed in ten hours, phagocytosis plays little part, for it is only after that time that leucocytes are found in large number. It is worthy of note, nevertheless, that the macrophages take up the gonococci with much greater avidity than do the finely granular polynuclears.

Phagocytosis by the eosinophiles (megoxycytes). — Occasionally the smears from certain animals contained a large number of coarsely granular polynuclear leucocytes, — eosinophiles. However, as has been shown by Opie,¹⁹ guinea-pigs vary greatly in the number of these leucocytes present in the exudate. Whether or not the eosinophiles, viz., the megoxycytes, ever act as phagocytes has been a moot question. It has recently been determined by Dudgeon and Ross¹⁸ that they may ingest staphylococci and *B. coli*, but they agree with other investigators in the opinion that their phagocytic activities are insignificant. At the autopsy of one of my guinea-pigs, dying twenty-four hours after inoculation, a large number of eosinophiles were found in the peritoneal exudate

and practically every one had taken up several gonococci. In a number of instances the cocci were certainly contained in vacuoles within the cytoplasm and undergoing digestive disintegration, — a convincing proof of phagocytic function.

Omentums. — Examining, now, the omentums from these animals, prepared and stained according to the method described in a previous article,¹⁷ I have found that as early as five minutes after inoculation there may be an extensive deposit of fibrin strands containing multitudes of gonococci, staining intensely, and also many leucocytes, viz., megoxycytes, small lymphocytes, macrophages and microxycytes. Until the complete destruction of the gonococci or the death of the animal, the macrophages far outstrip the polynuclears in activity of phagocytosis, a fact which Buxton has found to apply in regard to certain other bacteria. The elongated wandering cells between the bundles of connective tissue (the clasmatocytes of Ranvier, the "trailers" of Buxton and Torrey) have ingested in normal guinea-pigs only a moderate number or sometimes no cocci. On the surface of the omentums of animals dying in less than twenty hours there are many scattered intensely staining gonococci, but in the case of those which recover, practically all have disappeared in twenty-five hours. Whatever the outcome, the measure of phagocytosis is apparently the same, with the macrophages leading. Although there is a far greater microxycyte reaction in surviving than in dying animals this seems to be a secondary rather than a primary phenomenon. The guinea-pigs do not survive because there is a leucocyte reaction, but there is such a reaction because, through the early destruction of gonococci by other agencies, the organism is not weakened by widespread dissemination of toxin. For, as I have observed, in surviving animals an almost complete destruction of gonococci has taken place in the blood and peritoneal cavity before the microxycyte reaction becomes marked.

Cause of bacteriolysis. — Granting that the leucocytes take little part in the destruction of the gonococci within the peritoneal cavity, it may be assumed that the disintegration

is due to lytic action of the serous exudate. Furthermore, as I have found fresh normal rabbit serum strongly bactericidal for gonococcus, it seems probable that in these guinea-pigs an alexine brings about the lysis. Such an explanation conforms with the somewhat marked individual differences in susceptibility of guinea-pigs, for the serums from some rabbits have been found to be ten times as potent as that from others. If an autolytic enzyme plays an important part, as Flexner¹² has stated to be the case in similar experiments with *Diplococcus intracellularis*, one would not expect to find such decided individual variations in the rapidity with which the gonococci are destroyed. It seems reasonable to suppose, then, that guinea-pigs which recover do so because the body fluids contain sufficient alexine to kill the gonococci almost completely in less than ten hours; whereas, in the less resistant animals, the cocci are able to hold their own until the toxin has been carried to and acted on the vital organs.

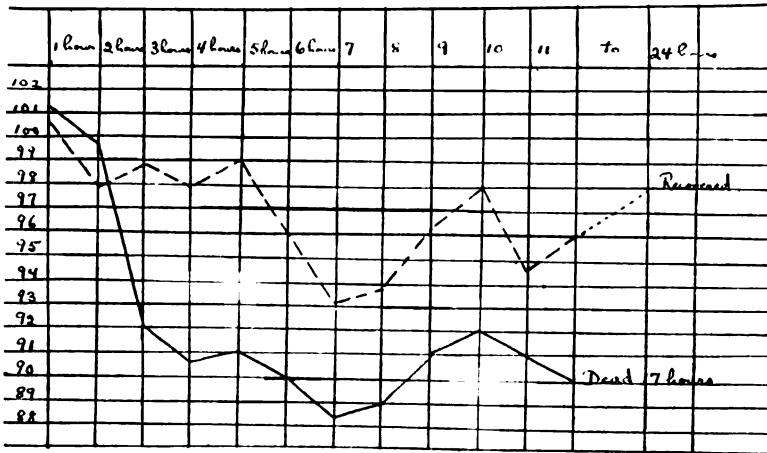
Infection of animals with the gonococcus. — As all investigators agree, there is no evidence that an infection, in the strict sense of the term, can be induced in animals. There is probably never more than a slight increase of the cocci in the body within a short time after the inoculation. Death occurs merely through the establishment of a toxemia. Although this is true, I have found that a living emulsion of culture A was about four times as toxic as the same amount of heated emulsion. Cantoni came to a similar conclusion, but Wassermann, Scholtz and Jundel have contended that living and dead gonococci produce identical results. In order to damage as little as possible the gonococcic autolytic enzyme a portion of an emulsion of living gonococci was rendered sterile (tested) by heating to 55° C. for one-half hour. In the following temperature chart (2) the curve of the animal receiving the heated part is compared with that of another pig of approximately the same weight which had been inoculated with a like amount of the living unheated part. The difference in the outcome is probably due to the living gonococci being more invasive and less readily

disintegrated than the dead ones; the toxin, accordingly, would be carried to vital points before being freed from the cocci by lytic agents.

TEMPERATURE CHART NO. 2.

Unbroken line indicates the temperature changes of a guinea-pig receiving an intraperitoneal injection of living gonococci.

Broken line is that of an animal receiving the same amount of emulsion heated to 55° C. $\frac{1}{2}$ hour.



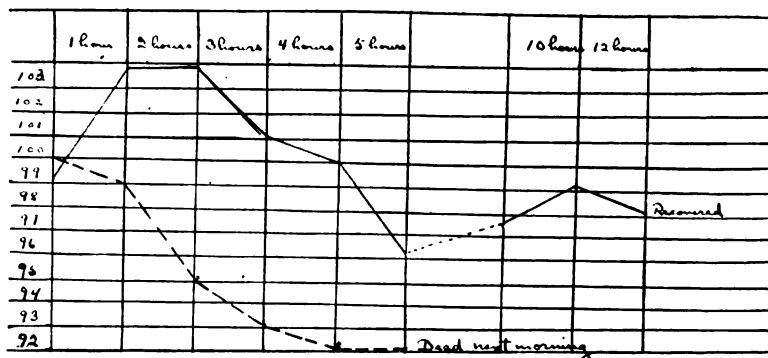
Mixed infections. — Occasionally a guinea-pig, apparently about to recover, would suffer a second drop in temperature and die in about fifty hours from the time of inoculation. In each instance it was found that death was due to a secondary infection. At autopsy, however, a good growth could be obtained from the peritoneal fluid and twelve to sixteen gonococci from a loop of heart blood. Leucocytes were comparatively scarce in the peritoneal fluid, but had ingested gonococci in preference to the other microörganism. The survival of the gonococci in these mixed infections, twenty-five hours beyond the usual period, may be due to the lowered temperature of the animal, the gonococcus thriving best at about 36° C., or more probably to the partial absorption of the lytic substances in the fluids by the contaminating bacteria.

Increase of virulence by passages. — Nicolaysen¹ was not able to increase the virulence of the gonococcus by passages. Negative results have also been reported by Scholtz³ and Wildbolz.² On the other hand Bruckner and his associates¹⁶ have described the acquisition by their strains of gonococcus of a marked augmentation of toxicity by passing them through rabbits, thus confirming similar previous experiments of Pinto.²⁰ To test the accuracy of these observations, culture A was passed through ten guinea-pigs. After one passage the temperature of the second animal dropped nine degrees in four hours, whereas with the same amount of original culture there was no fall within the same period in a control pig. After ten passages, one-fourth of the minimum certainly fatal dose of the original culture proved lethal for a pig, although not uniformly so. The virulence was certainly increased, probably through adaptive changes which render the gonococci resistant to the lytic agencies of the host, but the experiments were not sufficient in number to determine how far this may be continued. As was described in a previous article²¹ the culture became, through this treatment, much less agglutinable.

TEMPERATURE CHART No. 3.

Unbroken line represents the temperature curve of a guinea-pig inoculated intraperitoneally with one-half the certainly fatal dose of living gonococci A.

Broken line is that of an animal receiving one-half as much emulsion of strain A after passage through ten guinea-pigs.



III. The production of active immunity to gonococcus in guinea-pigs. — As the attempts to immunize rabbits and guinea-pigs by the use of ascitic broth cultures were uniformly unsuccessful on account of the free endotoxin in the medium, experiments have been carried out in which emulsions of twenty-four-hour ascitic agar cultures served as the immunizing agents.

Comparison of intraperitoneal and subcutaneous immunization. — Certain pigs were given weekly intraperitoneal inoculations of sublethal amounts of gonococcus emulsion and others subcutaneous vaccinations with the same dose. As might be expected, the former were protected against a lethal intraperitoneal dose by fewer treatments than the latter. Seven days after one immunizing injection the "intraperitoneal pigs" withstood a dose certainly fatal for control animals, whereas the "subcutaneous" pigs died in about thirty hours. After the second treatment, the outcome was the same, the "subcutaneous" pig showing even an increased susceptibility in comparison with the control. This apparent hypersensitivity may be due to the development of lytic antibodies in the fluids without a corresponding increase in tolerance to the gonotoxin. After four treatments, however, animals immunized subcutaneously withstood the certainly fatal dose and after seven treatments twice the lethal dose. This seemed to be the limit of resistance.

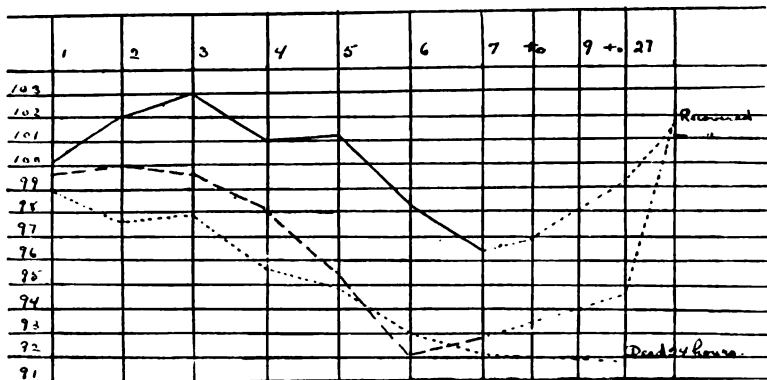
TEMPERATURE CHART No. 4.

Comparison of resistance of animals immunized intraperitoneally and subcutaneously. Both had received four immunizing inoculations.

——, Curve of animal immunized intraperitoneally.

- - -, Curve of animal immunized subcutaneously.

....., Curve of control normal animal.



With intraperitoneal immunization, the protection is not entirely specific on account of the local inflammation induced. Even ten days after the third treatment the leucocytes in the peritoneal fluid were far more abundant than in normal animals. In the following table are compared the percentages of the various forms of leucocytes in the peritoneal cavity; fifteen minutes after an intraperitoneal injection of normal saline (taken from Dudgeon and Ross¹⁸) I.; twenty-four hours after the first intraperitoneal inoculation with gonococcus, II.; and ten days after the third treatment, III.

	I.	II.	III.
Microcytes (neutrophils).....	1.4%	86%	1.6%
Megacytes (eosinophiles)	3%	1%	19.4%
Macrophages	1.4%	9%	32%
Small lymphocytes	94.2%	4%	47%

This comparative table shows graphically the gradual recovery of normal conditions after an intraperitoneal injection brought about through the almost complete destruction of the microxycytes by the macrophages and their replacement with megoxycytes and small lymphocytes.

As a far more specific immunity is obtained by subcutaneous vaccinations, this technic has been employed in all the following experiments:

Heated and living vaccines.—An investigation of the comparative protective properties of living and of heated (60° C.) vaccines has shown that each produced an immunity, but the animals treated with living emulsion of gonococcus gave evidence of protection earlier in the course of treatment and more completely in the end. Not only did they destroy living gonococci with greater rapidity, but they also attained a higher degree of tolerance for diffused gonotoxin, as is shown in the following temperature chart:

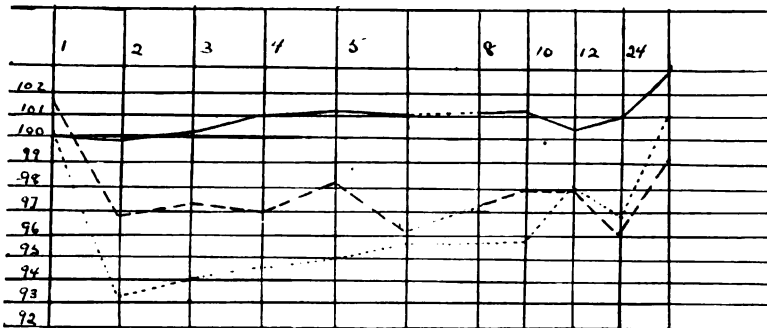
TEMPERATURE CHART NO. 5.

Comparison of "tolerance" for gonotoxin afforded by living and heated vaccines.

——, Curve of animal treated seven times with living vaccine and then given gonotoxin (filtrate from ascitic broth cultures) intraperitoneally, one cubic centimeter to fifty grams.

— — —, Curve of animal treated as above with heated vaccines and then given the same dose of toxin.

....., Curve of normal animal, after receiving the same amount of toxin.



Minimal vaccine dose.—To determine how small an amount of living vaccine may be given and yet obtain

protection against a fatal dose of culture A, pigs were injected subcutaneously with varying amounts of this culture. It was found that guinea-pigs receiving one-eighth of the fatal dose each time, in six weekly doses, or two-thirds of a culture in all, were protected, as was also an animal receiving one-twelfth of the fatal dose for the same period, or approximately one-half of a culture in all. No immunity, however, was conferred by treatments with one-sixteenth of a fatal dose or three-eighths of a culture altogether.

Tabulation of animals immunized to gonococcus. — With the purpose of ascertaining the rapidity and manner in which gonococci are destroyed in immunized animals a series of guinea-pigs were inoculated subcutaneously, during six to seven weekly intervals with one-half a culture. They were then given an intraperitoneal injection of one culture, the same dose that the series of normal animals received, and killed with chloroform at various intervals. The results of these experiments are outlined in the following table:

TABLE II.
Series of guinea-pigs immunized, subcutaneously, to gonococcus A, and then inoculated intraperitoneally with the same dose of culture A that the series of normal animals received.

Pig.	Time of Death or Chloroforming.	Culture, Peritoneal Fluid.	Culture, Heart Blood.	Smear of Peritoneal Fluid.	Omentum.	Remarks.
136.	15 minutes. Dead. Chloroforming began in 10 minutes.	30	Gonococci in clumps, almost entirely, 5 to 25 in a clump. A considerable number of macrophages and hyaline cells.	Great deposits of fibrin, filled with gonococci, which stain well, and also macrophages and microcytes. Some macrophages crowded with gonococci.	Omentum not rolled up. Suprarenal yellow.
205.	15 minutes.	1	Gonococci in small clumps and scattered. A few lymphocytes and microcytes.	Gonococci in fibrin stain pale, a few free clumps. Macrophages numerous, have taken up 8 to 20 gonococci. Microcytes rare.	Suprarenal yellow.
137.	30 minutes.	Good growth.	20	Fig. 137. Gonococci in clumps, stain very pale, ragged, not swollen. No leucocytes.	Fibrin crowded with gonococci which stain rather pale as do also free clumps. Macrophages crowded with gonococci. "Trailers" contain a few.	Omentum rolled up. Suprarenal yellow.
205a.	30 minutes.	0			
141c.	1½ hours.	Good growth.	15	Gonococci in clumps, stain pale. Very few leucocytes.	Only a few, pale, swollen gonococci. Great many "trailers," nearly every one contains a large number of cocci, many of these pale and swollen. Macrophages are also crowded with cocci for the most part.	Moderate amount of exudate in the peritoneal cavity.

TABLE II. — *Continued.*

Pig.	Time of Death or Chloro- forming.	Culture, Peritoneal fluid.	Culture, Heart Blood.	Smear of Peritoneal Fluid.	Omentum.	Remarks.
200.	1½ hours.	0	Fig 200. Gonococci in large and small clumps, many pale and disintegrated; few normal. Leucocytes very scarce.	Almost no gonococci to be seen, a few inside macrophages and trailers. A few pale clumps of gonococci.	Suprarenal slightly hyperemic.
201.	1¼ hours.	0			
138.	2 hours.	∞ Good growth.	1	Fig 138. Gonococci entirely in clumps, not many to be seen. Rather more leucocytes than usual, mostly small lymphocytes and hyaline cells. Several microcytes crowded with cocci.	Very few free gonococci. Some macrophages packed with cocci. Many microcytes, but are not phagocytic.	Suprarenal yellow.
202.	2 hours.	0			
76.	2 hours.	∞	2			
140.	4 hours.	∞ Good growth.	1	Very few gonococci and these in clumps. No leucocytes.	Free gonococci pale and swollen. Trailers contain a moderate number of cocci.	A great deal of peritoneal fluid. Suprarenal red.
107.	5 hours.	∞ Thin growth.	4	Fig 78. Gonococci have almost disappeared. Very few leucocytes. No microcytes. A few small lymphocytes and megacytes. Some megacytes have ingested cocci.	A few clumps of gonococci, which stain well. Some macrophages have taken large numbers of cocci. Moderate number of microcytes.	T. 66 3/5.
78.	5 hours.	∞	1			
101.	5 hours.	∞ Thin growth.	4			

141.	6 hours.	∞ Good growth.	6	Gonococci quite abundant, stain well, few in clumps. Very few leucocytes.	"Trailers" have taken up a great many gonococci.	This pig did not develop immunity. T. S6 very sick when chloroformed.
141a.	11 hours.	40	1	No gonococci to be seen. Many micrococci, which evidently arrived after destruction of gonococci. A few megococytes and macrophages.	Gonococci have entirely disappeared. Great micrococci reaction. Macrophages and "trailers" also abundant.	T. 96 3/5. Peritoneal fluid scanty. Suprarenal hyperemic.
69.	24 hours.	0	0	Great quantities of micrococci, a few gonococci still to be seen in macrophages and trailers.	Peritoneal fluid thick and viscid. Suprarenal yellow.
108.	25 hours.	0	0	Moderate micrococci reaction. Gonococci have practically all disappeared.	Peritoneal fluid scanty, thick. Suprarenal yellow.

The point of greatest interest displayed by this table is the rapidity and completeness with which the gonococci are killed in the blood. That there has been an initial rush of cocci from the peritoneal cavity to the vascular system, similar to that occurring in normal animals, is indicated by the number of cocci present in the heart-blood of the animals sacrificed in ten minutes. In almost every instance, however, the heart-blood after thirty minutes is practically sterile instead of swarming with cocci as in normal pigs. In two hours instead of the five hundred gonococci per loop of the normal pig after a similar inoculation, the blood is either sterile or contains only one or two microorganisms per loop. That agglutination of the cocci plays, in all probability, no part in blocking the "initial rush" through the lymphatics of the diaphragm and the thoracic duct is indicated by a comparison of the peritoneal fluids of the first two pigs; in the one killed in ten minutes, the gonococci were almost entirely in large clumps and yet there were thirty living cocci in a loop of heart-blood; in the other sacrificed in fifteen minutes, although there was little agglutination in the peritoneal fluid, only one gonococcus was recovered from a loop of the blood. It is evident, then, that after the same rapid absorption from the peritoneal cavity, there occurs a far quicker and more complete destruction of the gonococci in the blood of the immunized than in that of the normal animals. That this is due to the development of specific immune bodies there is every indication. As will be shown in a subsequent article, the serum of rabbits immunized to gonococcus contains a large amount of specific bactericidal immune bodies. The rapidity of the lytic action precludes the theory of its being due essentially to phagocytosis or to the presence of an autolysate.

Peritoneal fluid of immunized guinea-pigs.— Cultures from the peritoneal fluid show that it is practically sterile in eleven hours, denoting a more rapid bacteriolysis than with normal guinea-pigs. Contrary to the findings of Jundel," working with immunized rabbits, a typical Pfeiffer reaction of varying

intensity appeared in the peritoneal exudate of these immunized pigs. As early as ten minutes after inoculation, the gonococci were found in large clumps. In thirty minutes to one hour the cocci took the stain feebly, in some of the clumps only a few appearing normal, the others being reduced to mere bits of flocculent pale-staining matter. This disintegration continued until, after five hours, in thoroughly immunized animals not a gonococcus was to be seen except here and there in leucocytes, phagocytosis having served apparently as a means of temporary protection to the microorganism. It is noteworthy that in the case of an animal which failed to develop an immunity (141), there was an abundance of cocci in the peritoneal fluid in six hours and also no agglutination.

The leucocytes are practically the same in character and number as those met with in the peritoneal exudate of normal guinea-pigs. The period of leucopenia is also similar in time of advent and duration. In these immunized animals, however, when the tide of microcyte emigration sets in, there is little chance for phagocytosis as the gonococci have been almost completely killed and disintegrated by the increased lytic action of the peritoneal fluid. From the manner of the bacteriolytic process, it is probable that the rapid destruction of the cocci is due here, as in the blood, to specific antibodies and not to the presence of an autolysate.

Omentums of immunized animals. — The principal points of difference between these omentums and those of the normal series is the more active phagocytosis of the immunized, especially as regards the macrophages, a distinction which Buxton²² had also found to hold in work with other bacteria. Within fifteen minutes after inoculation the macrophages have ingested, in some instances, hundreds of cocci, whereas in normal guinea-pigs well-filled macrophages are not noticeable until about the two-hour stage. The trailers also take up the cocci more rapidly and in greater numbers, although this increased activity is not as marked as in the round macrophages. The microcyte emigration takes place as in normal animals, but in this case

after the destruction of the cocci is so far advanced that these leucocytes can take little or no part in the protective mechanism. Further evidence that the destructive processes are extracellular is presented by the fact that within thirty minutes after inoculation the multitudes of cocci caught up in the fibrin stain poorly, and whatever free cocci there may be are generally found clumped together, pale and swollen or reduced to granules.

In conclusion, then, it may be said that this protection afforded to guinea-pigs by subcutaneous vaccination is not primarily phagocytic or antitoxic in nature, but is due to a great increase in humoral bactericidal antibodies. Hence, through the rapid destruction of the gonococci, almost as large a dose of living gonococci may be borne as when a sterile emulsion is injected into a normal animal.

IV. Immunization experiments with meningococcus and *M. catarrhalis*. — With the purpose of determining whether the immunity described above is specific, a series of guinea-pigs were given six to seven weekly subcutaneous vaccinations with meningococcus and others with *M. catarrhalis* in practically the same dosage as that employed with gonococcus. Eight to ten days after the last treatment the animals were inoculated intraperitoneally with the same amount of living gonococcic emulsion as was used in the gonococcus-immunized series. As the important difference between the normal and gonococcus-immune animals was the rapidity with which gonococci are destroyed in the latter, these pigs were chloroformed at intervals up to two hours. The results of the experiments with meningococcus are given first.

TABLE III.
Series of guinea-pigs immunized, subcutaneously, to meningococcus and inoculated intraperitoneally with the same dose of gonococcus A as in the other series.

Fig.	Time of Chloroform.	Culture, Heart Blood.	Smear of Peritoneal Fluid.	Omentum.	Remarks.
209.	15 minutes.	5	Gonococci scattered, stain well. No leucocytes.	Gonococci in the fibrin stain well. Trailers have taken up a good many cocci, 5 to 20.	Suprarenal yellow.
169.	30 minutes.	22	Gonococci not clumped, stain well. Leucocytes quite abundant and actively phagocytic. Macrophages stuffed with cocci, for more. Microcytes have ingested 20 to 30 in many instances.	Gonococci stain well and appear normal. Poor preparation.	Suprarenal yellow.
207.	30 minutes.	325	Gonococci not agglutinated. Stain well. No leucocytes.	Macrophages and trailers have taken up gonococci as freely as in gonococci-immune animals.	Suprarenal yellow.
210.	1 hour.	122	Fig 171. Gonococci stain very well; not clumped; swollen and pale as in gonococci-immune animals. Very few leucocytes.	Gonococci in the strands of fibrin stain deeply. Macrophages stuffed with cocci. Trailers contain only a few.	Suprarenal yellow.
170.	1 hour.	200			
171.	1 hour.	6	Leucocytes much more abundant than usual and actively phagocytic. Microcytes and macrophages. Gonococci scattered, stain well.	Gonococci free and in fibrin stain deeply. Nearly all macrophages gorged with cocci. Trailers also have taken up a good many.	Suprarenal slightly hyperemic. Reduction of cocci in blood is possibly due to a local inflammation of the peritoneum.
211.	1½ hours.	240	Gonococci stain well, not clumped or pale. No leucocytes.	Macrophages well filled with gonococci which stain intensely.	Suprarenal yellow.
206.	2 hours.	350	Gonococci not agglutinated, nor disintegrated. No leucocytes.	Gonococci in fibrin and free, stain well. Macrophages filled with cocci. Few microcytes.	Suprarenal hyperemic.

Cultures from the heart-blood of these meningococcus-immunized animals indicate the presence of a condition practically identical with that which we have encountered in normal animals. Instead of the blood being nearly or quite sterile after one hour as in gonococcus-immunized animals, there were, with one exception, from one to three hundred cocci in the loop. In the pig, in which the count was reduced to six, an unusual number of actively phagocytic leucocytes in the peritoneal fluid suggested a possible local inflammatory condition. Except in this instance there was no evidence of a more rapid destruction of gonococci in the blood of meningococcus-immunized than in normal animals. Smears from the peritoneal exudate also presented marked differences from those of gonococcus-immunized animals. In no instance was there a definite clumping of the gonococci, nor did they lose their staining properties and disintegrate, as was the case in the peritoneal fluid of the animals immunized to gonococcus. In other words, there was no evidence of a Pfeiffer reaction in this series of experiments, but how far such a test may prove useful in distinguishing between gonococcus and meningococcus has not, as yet, been determined. There was, however, one point of similarity between the gonococcus- and the meningococcus-immunized animals, and that lay in an equal activity of phagocytosis. This was especially evident in the examination of the omenta, for the macrophages and "trailers" of the meningococcus-immune have taken up great quantities of gonococci even fifteen minutes after inoculation. The gonococci, it should be added, stained intensely both in the fibrin and scattered on the surface of the omentum.

Guinea-pigs immunized to *M. catarrhalis*. — As a further control of the specificity of the phenomena occurring in these gonococcus-immunized animals, the results with guinea-pigs similarly immunized with *M. catarrhalis* and finally inoculated intraperitoneally with gonococcus are of interest.

TABLE IV.
*Series of guinea-pigs immunized, subcutaneously, to M. catarrhalis and then inoculated intraperitoneally with the same dose of living gonococcus
 A as before.*

Fig.	Time of Chloroforming.	Culture, Heart Blood.	Smear of Peritoneal Fluid.	Omentum.	Remarks.
173.	30 minutes.	225	Gonococci scattered, no agglutination, stain well. Few leucocytes, hyaline cells.	Great many "trailers;" majority have not ingested any cocci. Very few micrococci and little phagocytosis. Gonococci in fibrin stain dark.	Suprarenal yellow.
120.	1½ hours.	150	Gonococci abundant, scattered, stain well. No leucocytes.	Trailers have taken up many cocci about like normal. Gonococci stain intensely.	Suprarenal yellow.
174.	2 hours.	105	Gonococci scattered, stain well. More leucocytes than usual. Micrococci have taken up few cocci. Some macrophages well filled, others empty.	A good many patches of fibrin containing quantities of micrococci and gonococci; the latter stain well. Macrophages have taken up large quantities of cocci as have also a number of trailers.	Suprarenal slightly hyperemic.
121.	2 hours.	145	Gonococci not at all agglutinated, stain well. Very few leucocytes.	Not many gonococci to be seen. These stain well. Macrophages contain only a few or no cocci.	

As in the meningococcus-immunized animals, there was also in this series no greater destruction of gonococci in the blood than in normal guinea-pigs; nor was there the slightest indication of a Pfeiffer reaction in the peritoneal fluid. Although this series possibly contained an insufficient number of experiments for a definite conclusion, the evidence indicated that there was no greater or more rapid phagocytosis in catarrhalis-immunized than in normal animals.

Inter-protective immunization between gonococcus and meningococcus. — Wollstein¹¹ has described an inter-protective immunization between gonococcus and meningococcus, but, as she suggested, these results may be due to the fact that the immunizing inoculations were given intraperitoneally. My experiments lead me to the opposite conclusion, at least as regards gonococcus. Whereas, four subcutaneous injections of gonococcus, one cubic centimeter of standard emulsion to four hundred grams of weight, have invariably protected a guinea-pig against an ordinarily fatal intraperitoneal dose of living gonococcus (all four animals surviving, where the controls died), two out of three guinea-pigs similarly immunized with meningococcus and two out of four vaccinated with *M. catarrhalis* died, when given intraperitoneally a like dose of gonococcus. None of the animals, too, treated with meningococcus or with *M. catarrhalis*, exhibited any definite signs of immunity as they suffered as rapid a drop in temperature and the same symptoms of distress as the normal guinea-pigs. This peculiarity, as regards temperature, is demonstrated in the following chart.

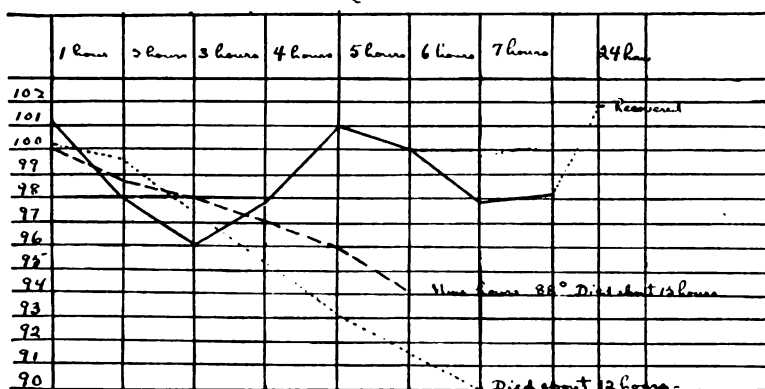
TEMPERATURE CHART NO. 6.

Comparison of gonococcus-, meningococcus-, and *M. catarrhalis*-immunized guinea-pigs.

—, Curve of animal treated with gonococcus vaccine (four weekly subcutaneous treatments) and then inoculated intraperitoneally with living gonococcus emulsion, one cubic centimeter to seventy-five grams.

- - -, Curve of animal similarly treated with meningococcus vaccine (four times) and then given gonococcus, one cubic centimeter to one hundred grams.

...., Curve of catarrhalis-immunized animal (six times) given gonococcus, one cubic centimeter to one hundred grams.



SUMMARY.

The toxin of gonococcus is derived solely from the bodies of dead and disintegrated gonococci.

That culture medium in which there is the greatest amount of growth, and hence the most active disintegration of gonococci, contains the largest amount of toxin, regardless of the constitution of the medium. No immunity to gonotoxin could be induced in guinea-pigs.

Certain guinea-pigs, after several injections of gonotoxin, became hypersensitive and died suddenly with symptoms suggestive of anaphylaxis.

Strains of gonococcus differ greatly in toxicity.

In guinea-pigs destined to recover after an intraperitoneal inoculation of an emulsion of living gonococci, the number of the cocci are greatly decreased in ten hours, but the peritoneal fluid of those dying in less than eighteen hours contain

great quantities of living gonococci. After twenty hours, whatever the outcome, there are few or no gonococci alive in the peritoneal exudate. From the heart-blood after intraperitoneal inoculation, gonococci were recovered in greater or less abundance up to twenty-four hours. In five minutes after inoculation there were fifty in $1/140$ th of a cubic centimeter, indicating a rapid absorption from the peritoneal cavity. The maximum number, five hundred, in this amount of blood was found in two hours. From this time there is a rapid decrease in number in recovering animals, until in ten hours the blood is practically sterile. In animals up to twenty hours, however, there may be a large number in the blood.

The leucopenic stage of the peritoneal fluid, after intraperitoneal inoculation, extends from the second to the seventh hour. Macrophages in the peritoneal exudate ingest gonococci with much greater avidity than the polynuclear leucocytes. Phagocytosis, however, plays little part in the destruction of the gonococci.

Eosinophilic leucocytes occasionally ingest a small number of gonococci and digest them in vacuoles.

On the omentum, also, the macrophages are more actively phagocytic than the polynuclears. The degree of phagocytosis is apparently the same whether the animal dies or recovers.

Guinea-pigs recover because the body fluids contain sufficient alexine to cause the destruction of the gonococci in about ten hours.

An infection with gonococcus does not take place in guinea-pigs, but they die because of the establishment of a toxemia. Living emulsions of the strain of gonococcus employed were about four times as toxic as the same amount of emulsion rendered sterile by heating to 55° C. for one-half hour.

If a mixed infection occurs, gonococci may be found alive in the peritoneal exudate and blood fifty hours after inoculation.

This strain of gonococcus was increased in virulence for guinea-pigs, by passage through these animals.

A certain degree of specific immunity to emulsions of living gonococci may be induced by vaccinations with the same.

Guinea-pigs immunized by intraperitoneal inoculations were protected against a lethal intraperitoneal dose by fewer treatments than by subcutaneous vaccination.

Animals immunized subcutaneously with living vaccines were protected earlier in the course of treatment and more completely in the end than when heated vaccines were employed.

An animal receiving one-twelfth of the fatal dose, for six weekly subcutaneous treatments, was protected against the certainly fatal dose for normal pigs.

In a series of guinea-pigs immunized by subcutaneous vaccinations, gonococci, absorbed from the peritoneal cavity, were very rapidly destroyed in the blood. The heart-blood was practically sterile thirty minutes after intraperitoneal inoculation. This rapid destruction is, in all probability, due to specific immune bactericidal bodies.

A Pfeiffer reaction of varying intensity occurred in the peritoneal fluid of these immunized animals.

The round macrophages on the surface of the omentums of the immunized animals ingested gonococci much more rapidly and in greater number than those of normal animals. This was also true, in a less degree, of the "trailers."

In a series of guinea-pigs immunized to meningococcus and also in another immunized to *M. catarrhalis*, practically no protection was developed against gonococcus. The gonococci were not destroyed more rapidly in the blood than in normal animals, nor was there a Pfeiffer reaction in the peritoneal fluid. The macrophages on the surface of the omentum of meningococcus-immunized pigs ingested gonococci as rapidly as did those of gonococcus-immunized animals. The macrophages of the *catarrhalis*-immunized animals, however, displayed no greater phagocytic activity than those of normal guinea-pigs.

REFERENCES.

1. Nicolaysen. *Centralb. f. Bakt.*, 1897, xxii, 305.
2. Wassermann. *Zeitschr. f. Hygiene*, 1898.
3. Scholtz. *Archiv. f. Dermatol.*, 1899, xlix.
4. Christmas. *Annales de Inst. Pasteur*, 1900, xiv, 331.
5. Vannod. *Centralbl. f. Bakt.*, 1907, xliv, 10 and 110.
6. Cantoni. *Riforma Medica*, 1899, xv.
7. Thalmann. *Centralb. f. Bakt.*, 1902, xxxi.
8. Teague and Torrey. *This Journal*, 1907, xvii, 223.
9. Jundel. *Hygeia*, 1900, lxii, 604.
10. Moltschanoff. *Munchen. med. Woch.*, 1899, No. 31.
11. Wollstein. *Journ. Exper. Med.*, 1907, ix, 588.
12. Flexner. *Journ. Exper. Med.*, 1907, ix, No. 2.
13. Detweiler. *Trans. Assoc. Amer. Physicians*, 1902.
14. Tracy. *This Journal*, 1907, xvi, 307.
15. Wildbolz. *Archiv. f. Dermat. u. Syph.*, 1902.
16. Bruckner, Cristeanu and Cinca. *Comptes. r. Heb. Soc. de Biologie*, 1906, Nos. 18 to 23.
17. Buxton and Torrey. *This Journal*, 1906, xv, 1.
18. Dudgeon and Ross. *Trans. Path. Soc.*, London, 1906, lvii.
19. Opie. *Trans. Assoc. Amer. Physicians*, 1904.
20. Pinto. *Journ. de Phys. et de Path. generale*, 1904, vi, 1058.
21. Torrey. *This Journal*, 1907, xvi, 329.
22. Buxton. *This Journal*, 1907, xvi, 251.

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BACTERIOLYSIS OF THE GONOCOCCUS AND OF THE MENINGOCOCCUS WITH NORMAL AND SPECIFIC IMMUNE RABBIT SERUMS.*

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In previous communications,^{1, 2} the writer has mentioned the fact that the serums of rabbits immunized to the gonococcus are rich in specific bactericidal immune bodies. The experimental evidence, which both justifies this assertion and also discloses the same attributes for the meningococcus, is to be found in the following pages.

It is generally agreed that the Neisser-Wechsberg plating method^{3, 4} provides an accurate means of determining, quantitatively in vitro, the bactericidal potency of an immune serum. This technic has been used by Stern and Krote,⁵ Laubenheimer,⁷ Krote and Steinberg⁶ and others in the detection of specific bacteriolysins in the serum of typhoid patients acutely ill or convalescent; by Töpfer and Jaffé⁸ and by Neufeld and Hüne⁹ in studies of the anti-bodies in the blood of patients sick or recovering from typhoid and paratyphoid as compared with those produced by experimental inoculations; by Wright,¹⁰ by Shiga¹¹ and others in determinations of specific blood changes in man following protective vaccinations. These and various other investigations have shown that the typhoid, the paratyphoid and the dysentery bacillus stimulate the production of specific

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bactericidal immune bodies, as revealed by in vitro tests, as do also *V. cholerae* and certain other vibrios. On the other hand, repeated attempts to demonstrate, by this method, the presence of these anti-bodies in serums immune to such Gram-positive cocci as pneumococcus, streptococcus and staphylococcus have led to negative results. As regards the Gram-negative diplococci, however, no experiments in accordance with this method have been reported, save a brief note by Neisser⁴ in reference to the gonococcus and the preliminary statements of the writer. The positive results for the gonococcus and the meningococcus, described herein, aside from a purely scientific interest, may be of significance in connection with the serum-therapy of the diseases which they incite.

The susceptibility of these diplococci, especially the gonococcus, to unfavorable physical and chemical conditions renders accurate bactericidal tests with them a pathway beset with pitfalls. These experiments have been conducted for a year and a half, and a considerable part of that time has been employed in overcoming difficulties and developing a technic which, if rigorously followed, would lead to uniform and consistent results. As the method adopted is somewhat complicated and contains many details, which it is essential to observe, the various steps are described at length.

Methods. — According to the Neisser-Wechsberg technic a few drops of broth are added to each tube containing the dilutions of activated immune serum, seeded with a definite amount of bacteria, and also to the controls. Hüne¹² found that for *B. typhosus* and *V. cholerae* the optimum proportion of broth was one part to six parts of the whole mixture. Although this degree of enrichment is quite sufficient to sustain the vitality of these microorganisms, it is inadequate for the gonococcus. Experiments showed that each tube must be provided, not only with broth, but also with a definite amount of serum. The following mixture was finally adopted for the tube containing necessarily the minimum enrichment, viz.: the control of the seeding of culture. Serum broth was first prepared by adding one cubic centimeter of inactivated normal rabbit serum to fifteen cubic centimeters of "Thalmann's" broth (reaction + 1 to phenolphthalein). Of this rabbit serum broth, one cubic centimeter was added to three cubic centimeters of .9 per cent normal saline

solution. In this mixture, with serum broth present in the proportion of one to four, the strains of the gonococcus employed generally increased slightly in four to five hours, decreasing rarely and in negligible degree, and in eighteen hours multiplied sufficiently to cloud the mixture. The criticism might be raised that the use of this amount of inactivated normal serum for enrichment would influence the results obtained, by the introduction of other elements into the experiments, such as extraneous intermediate bodies. As regards the tests with the reactivated immune serums, a number of points, as for example the well-defined and consistent non-killing post-zones, show that this factor is of no significance. Certain control experiments with fresh normal serums, however, seemed to indicate that their bactericidal titer for the gonococcus may be increased, in slight degree, by the presence of this inactivated normal serum, but not so far as to interfere with the comparative value of the tests.

As is indicated in the following chart (Table I.) the two constant elements in all the mixtures employed in these tests are the one cubic centimeter of serum broth and the one-half cubic centimeter of the emulsion of the diplococcus in saline solution. The other elements (diluted active or inactive immune serum and diluted fresh normal serum) were added in one-cubic-centimeter amount, as the purpose of the test required, and .9 per cent salt solution in amount sufficient to bring the whole to four cubic centimeters. The object of this chart is the avoidance of needless repetition and by reference to it the constituents and their proportions in any test mentioned in the following tables may be determined.

TABLE I.

Chart showing the elements and their proportions as they occurred in tests mentioned in subsequent tables.

Mixtures.	NaCl Solution .9 Per Cent.	Inactivated Immune Serum Dilution.	Fresh (Active) Immune Serum Dilution.	Serum Broth.	Fresh Normal Serum (Complement) Dilution.	Seeding Emulsion.	Total.
Test of fresh (active) immune serum.....	1.5 cc.	—	1 cc.	1 cc.	—	.5 cc.	4 cc.
Test of heated and reactivated immune serum.....	.5 "	1 cc.	—	" "	1 cc.	" "	" "
Controls. { Test of inactivated immune serum without complement	1.5 "	" "	—	" "	—	" "	" "
Test of fresh normal serum (complement).....	" "	—	—	" "	1 cc.	" "	" "
Test of emulsion of bacteria used in seeding ..	2.5 "	—	—	" "	—	" "	" "

The immune serums were produced in rabbits according to methods which have been described in previous articles. In general the animals had received seven to nine inoculations at the time of bleeding, which was performed eight to ten days after the last inoculation. The serums were inactivated by heating in a water bath at 55° to 56° C. for one-half hour and kept on ice. No preservative was added and no trouble encountered from contamination.

For complement, a normal rabbit was bled from the ear about eighteen hours before the experiment and the clotted blood kept on ice until the time of use. The serum was diluted and added to the tubes as rapidly as possible. If a large number of tubes are to be supplied, the flask containing the diluted fresh normal serum should be packed in ice — a procedure which was found desirable in connection with the "fixation of complement" experiments.¹⁶

The cultures used in these tests have all been under cultivation on ascitic agar for over two years. The differences in virulence and rapidity of multiplication of the gonococcus strains were not marked. Mention is made of this fact because Hüne¹² has stated that virulent strains of *B. typhosus* and *V. cholerae* multiply faster and are more resistant than the avirulent to destructive agents in serum. It is probable that uniform results with freshly isolated cultures of the gonococcus would be obtained with great difficulty, because of their marked sensitiveness to unfavorable conditions.

A standard emulsion of the diplococci for seeding the tubes was obtained in the following way. Growth from an eighteen-hour ascitic agar culture was taken in amount sufficient to cloud very slightly five cubic centimeters of saline solution. It was necessary to vary the degree of cloudiness for various strains, on account of differences in the number of living cocci in a like amount of growth. Such variations precluded the seeding of the tubes with a fixed amount (by weight) of growth. This five-cubic-centimeter emulsion was then poured into a flask containing two hundred cubic centimeters of sterile .9 per cent saline solution, and the whole thoroughly shaken. Each half cubic centimeter would then contain from forty thousand to two hundred thousand diplococci. The process of seeding the tubes should not occupy more than ten minutes, as after that period the diplococci begin to die out more or less rapidly.

In making the mixtures, the required amount of saline solution (sufficient to bring the completed mixture to four centimeters) was placed in a five-inch test-tube. Next was added the serum broth, then the dilution of the serum to be tested and the complement and finally one-half cubic centimeter of the emulsion of diplococci. After each addition the tube was well shaken. The tubes containing the various mixtures were then placed in an incubator at a temperature of 36° to 37° C.

As it is necessary to use some such medium as ascitic agar in plating, the process is rather more complicated than is plating as ordinarily conducted. Agar, made according to the method of Thalmann and titrated

+ 1.2 to phenolphthalein, was tubed in eight-cubic-centimeter amounts. Just before the experiment these were melted and cooled to about 50° C., and to each was added three cubic centimeters of ascitic fluid heated to the same temperature. Until used the ascitic agar tubes were placed in a water bath with a temperature sufficient to prevent solidification. The mixtures were plated out after an incubation of four and one-half hours. A tube was thoroughly shaken and one-half cubic centimeter withdrawn and placed in a sterile petri dish. Over this was quickly poured the eleven cubic centimeters of ascitic agar, which had been cooled until only slightly warm to the hand, and the whole thoroughly mixed. For each tube in the experiment a fresh sterile one-half cubic centimeter pipette was used. It would seem that the plating out of one-half cubic centimeter from each of the seeded tubes, instead of a certain number of drops, as recommended by Neisser, is conducive to greater accuracy.

After the plates had solidified, they were placed (inverted) in the incubator, as were also the tubes containing what remained of the mixtures. Although in eighteen to twenty-four hours the colonies at times attained a sufficient size to permit counting, it should be deferred until forty-eight hours, as the stronger dilutions of the immune serums often exercise a marked inhibitory influence. Plates made from these tubes, apparently almost sterile in twenty-four hours, may show a multitude of colonies on the second day. Hüne¹⁸ and others have encountered like inhibitory conditions in these plating experiments. Plates containing less than ten thousand colonies were counted with a reasonable degree of accuracy, above that number the figures given are in a larger measure approximate.

In investigations of this character controls are of the utmost importance. These should include: (1) a control tube indicating the number of bacteria seeded, from this tube platings should be made at once and again after the period of incubation to detect any numerical change in bacterial content; (2) a control tube indicating the degree of destructive action of the amount of fresh normal serum, alone, used to reactivate the immune bodies; (3) a control tube showing the degree of inhibitory action of the strongest dilution of inactivated immune serum alone; (4) plates controlling the sterility of the various elements in the experiment. Controls of sterility are not recorded in the tables as the inactivated serums and the fresh normal serums proved almost without exception to be sterile. The detection, in fact, of any contamination, occurring during the course of these experiments, was not difficult, thanks to the characteristic appearance of the gonococcus and the meningococcus colonies.

Bacteriolysis with the fresh serum of normal rabbits. — Many investigators have found that normal rabbit serum may be quite strongly bactericidal for certain bacilli and vibrios. This lytic action may be manifest for *B. typhosus* and *B. paratyphosus* in as high dilutions as 1-10 to 1-30,

and for *V. cholerae asiaticæ* at still greater weakening (Töpfer and Jaffé,⁸ Buxton,¹⁸ Hüne¹⁹), but for *Micrococcus gonorrhæ* and *Diplococcus intracellularis meningitidis* no accurate quantitative estimations have hitherto been reported. It is evident, however, that in order to conduct tests in the reactivation of serums immune to these diplococci, their degree of susceptibility to fresh normal serum itself should be determined.

As has been frequently reported, apparently normal rabbits differ greatly in the bactericidal potency of their fresh serums. Accordingly, it seemed advisable to set aside a number of normal animals and to titrate accurately the strength of their complement. Not only, however, were there found to be differences in the serum potency of individual rabbits, but also changes occurred from time to time in the same animal. During the fall, winter, and spring these variations were not marked, but with the advent of the hot summer months a decrease in strength of one-half to one-third was not uncommon. The irregularities at this time became so marked that the successful reactivation of immune serum was a matter of great difficulty. Cohn¹⁴ has also noted that fresh normal serum is more strongly bactericidal in winter than in summer. This is due, he thinks, to the temperature of the air and not to food or other factors.

In the following table are given the results with the fresh serum of two normal rabbits: one (Rabbit No. 4) with a low bactericidal titer; and another (Rabbit No. 5) in which it is rather high.

TABLE II.

Bactericidal action of fresh normal rabbit serum on six strains of the gonococcus and on one strain of the meningococcus. Rabbit No. 4, weak; No. 5, strong.

Dilutions of Fresh Normal Rabbit Serum.	Actual Dilution of Serum in Mixture.	Gonococcus Culture A, Plated in 4 hrs.	Culture B.	Culture C.	Culture G.	Culture H.	Culture I.	Meningococcus.
1. Rabbit No. 4, 1-2	1-8	0	1	2	125	12	100	1
2. " " 1-10	1-40	35	230	40	300	1,900	8,500	200
3. " " 1-25	1-100	700	3,150	120	25,000	20,000	20,000	3,000
4. " " 1-50	1-200	10,500	20,000	30,000	25,000	17,000	20,000	10,000
5. " " 1-80	1-320	25,000	20,000	35,000	25,000	20,000	20,000	10,000
6. " " 1-150	1-600	25,000	30,000	—	—	—	—	—
7. Seeding, Control	—	25,000	30,000	35,000	25,000	20,000	20,000	10,000

Control seeding, plated at once. A, 25,000; B, 40,000; C, 35,000; G, 15,000; H, 20,000; I, 20,000; meningococcus, 10,000.

Dilutions of Fresh Normal Rabbit Serum.	Actual Dilution of Serum in Mixture.	Gonococcus Culture A, Plated in 4 hrs.	Culture B.	Culture C.	Culture G.	Culture H.	Culture I.	Meningococcus.
1. Rabbit No. 5, 1-2	1-8	0	0	0	0	1	0	50
2. " " 1-10	1-40	0	0	0	300	7	160	250
3. " " 1-25	1-100	7	1	210	25,000	2,800	1,200	1,050
4. " " 1-50	1-200	35	70	400	28,000	3,150	20,000	2,800
5. " " 1-80	1-320	1,120	150	1,500	28,000	25,000	20,000	24,000
6. " " 1-150	1-600	10,000	30,000	30,000	—	20,000	—	—
7. Seeding, Control	—	22,000	30,000	35,000	28,000	20,000	20,000	35,000

Control seeding, plated at once. A, 22,000; B, 30,000; C, 35,000; G, 17,000; H, 20,000; I, 17,000; meningococcus, 15,000.

Two points in this tabulation are noteworthy. First, that these diplococci, in general, are decidedly susceptible to the bactericidal action in vitro of fresh normal serum, and second, that certain strains are much more sensitive than others. The more susceptible strains are A, B, and C; the hardier, G, H, I. This grouping is significant from the fact that previous experiments in agglutination¹⁵ and in "fixation of complement"¹⁶ have indicated the diversity of these two

sub-groups. The susceptible strains apparently are ten times more readily destroyed by fresh normal rabbit serum than *B. typhosus*, culture A suffering some destruction with No. 5 serum diluted 1-150, and with a still more potent rabbit serum at 1-300. The culture of meningococcus, used in these experiments, reacted to fresh normal serum in a way similar to the more resistant gonococcal strains G, H, and I.

Fresh immune serums. — Theoretically, one would expect that a serum immune to the gonococcus, when tested fresh in vitro and without extraneous complement, would have approximately the same bactericidal titer for this diplococcus as the average fresh normal serum. The accompanying tabulation (Table III.) of the results of a series of tests with the fresh serum from a rabbit undergoing inoculations with culture A substantiates this expectation. In this and the following tables it should be observed that the dilution of the serum, indicated, is really only one-fourth of the actual dilution in the experiments, because of the other elements in the mixtures (saline solution, serum broth, etc.).

TABLE III.

Bactericidal action of serum drawn at various periods from a rabbit undergoing inoculations with gonococcus A. Serum tested fresh.

Dilutions of Serum from Rabbit 386.	Normal Serum from Rabbit 386.	Serum drawn 6 Days after First Inoculation.	Serum drawn 8 Days after Third Inoculation.	Serum drawn 8 Days after Fifth Inoculation.	Serum drawn 9 Days after Seventh Inoculation.	Serum drawn 7 Days after Ninth Inoculation.	Serum drawn 10 Days after Fifteenth Inoculation.
1. 1-2	—	—	—	0	0	0	0
2. 1-5	—	1	0	0	0	1	0
3. 1-25	60	15	31	3,500	0	980	90
4. 1-50	140	210	1,560	11,000	0	6,790	15,800
5. 1-75	1,050	12,000	20,000	12,500	2	9,000	35,000
6. 1-100	11,000	25,000	28,000	15,000	22	10,000	25,000
7. 1-125	20,000	25,000	30,000	19,000	910	16,000	35,000
8. 1-150	25,000	25,000	30,000	19,000	5,600	17,000	35,000
9. 1-200	25,000	25,000	30,000	19,000	2,380	16,000	—
10. Control seeding	25,000	25,000	30,000	19,000	25,000	25,000	35,000

Up to the fifth inoculation there occurred a progressive drop in the specific bactericidal action of this fresh immune serum, a sudden rise after the seventh, followed by irregular increase and decrease to the fifteenth inoculation. It seems reasonable to suppose that these variations are due to changes in the complement content of the rabbit's blood rather than to fluctuations in the number of immune bodies. This is, evidently, true from the fact that immune bodies were found to be present in great abundance in the inactivated serum, drawn after the fifth inoculation, yet the killing titer of this serum, when active, was only one-fourth that of the normal. Again, the fresh serum after the seventh inoculation was far more bactericidal than after the fifth, and yet contained rather fewer immune bodies. According to the experiments of Buxton,¹⁷ fresh immune typhoid and paratyphoid serum may or may not be specifically bactericidal, depending to a certain extent on the number of the inoculations. He found that after the first inoculation the "killing zone" might rise rather higher than with the normal serum, but with succeeding inoculations a prezone appeared, in which there was no bactericidal action, and this broadening finally reached the non-killing post-zone. Thus, after the fourth or fifth inoculation the fresh immune serum was not specifically bactericidal in vitro at any dilution. With fresh antigenococcic serum, apparently, the results are quite different, as no prezone has appeared at any stage of immunization and there is always a more or less extensive killing zone. In these respects this serum resembles fresh cholera immune serum. The low specific killing titer of these fresh immune serums is certainly not due to a poverty of immune bodies, but rather to the fact that in the graded dilutions a point is soon reached at which the complement is depleted to such an extent that it is entirely deflected by the plethora of immune bodies.

The serum of a rabbit inoculated nine times with meningococcus (Table IV.), when tested fresh, had certainly no stronger specific bactericidal action than normal serum. It

also reacted on gonococcus A to the same degree as fresh normal serum.

TABLE IV.

Bactericidal action of fresh meningococcic serum on the meningococcus and the gonococcus A.

Dilutions of Fresh (Active) Meningococcic Serum.	Culture Meningococcus, Plated in 4½ Hours.	Tubes in 24 Hours.	Culture Gonococcus A, Plated in 4½ Hours.	Tubes in 24 Hours.
1. 1-5	350	No growth.	50	No growth.
2. 1-10	280	" "	4	" "
3. 1-25	4,900	" "	9	" "
4. 1-50	3,500	Growth.	200	" "
5. 1-100	12,000	"	40,000	Growth.
6. 1-250	13,000	"	40,000	"
7. 1-500	13,000	"	40,000	"
8. 1-1,000	12,500	"	40,000	"
9. Control seeding ..	30,000	"	40,000	"

Control seeding, plated at once. Meningococcus, 5,000; gonococcus A, 20,000.

Reactivation of immune serums. — The Neisser-Wechsberg method for the quantitative determination of specific bactericidal immune bodies consists essentially in the use of graded dilutions of the inactivated immune serum in conjunction with a fixed amount of complementing serum. By far the most difficult feature in such experiments with the gonococcus is the determination and the employment of the proper amount of complement. If complement is present in too great abundance the specific action of the inactivated immune serum will be masked by the bacteriolysis of the fresh normal serum itself, while if the amount be too small the immune bodies will fail of reactivation. As Neisser and others have proved, for the optimum specific bactericidal action, complement and amboceptor must be in right relation.

Experiment to demonstrate the proper relation of complement and immune body. — Although von Nadoleczny¹⁸ has found that the bactericidal properties of a fresh normal serum may stand in no direct relation to its complementing properties, in these experiments the one seemed to serve as a useful index of the other, viz., it was found necessary in reactivation to use a larger dosage of a normal serum weak in bactericidal properties for the gonococcus than of one strongly bacteriolytic. In order to determine, then, the amount of a fresh normal serum which would reactivate a given number of immune bodies and yet not, in itself, destroy more than a negligible number of the gonococci seeded, the following experiment was performed. To a constant dilution of inactivated immune serum and seeding of gonococcus A were added graded dilutions of fresh normal serum. Parallel with this another series was run with the same dilutions of complement, but with the immune serum replaced by normal saline solution. The results are given in Table V.

TABLE V.

Experiment to show the optimum ratio of complement to immune body.

Inactivated Immune Serum from Rabbit No. 361 Inoculated with Gonococcus A.	Complement Serum from Normal Rabbit No. 6.	Culture.	Colonies in 4½ Hours.	Tubes in 24 Hours.	Controls of Complement used in Test. Same Dilutions.	Colonies in 4½ Hours.	Tubes in 24 Hours.
1. 1-1,000....	1/40 cc.	A.	1	Very slight growth.	1/40 cc.	40	Good growth.
2. 1-1,000....	1/45 "	"	1	Very slight growth.	1/45 "	560	" "
3. 1-1,000....	1/50 "	"	2	Slight growth.	1/50 "	2,800	" "
4. 1-1,000....	1/55 "	"	1	Fair growth.	1/55 "	4,200	" "
5. 1-1,000....	1/60 "	"	4	" "	1/60 "	10,000	" "
6. 1-1,000....	1/65 "	"	17	" "	1/65 "	14,080	" "
7. 1-1,000....	1/70 "	"	70	" "	1/70 "	15,000	" "
8. 1-1,000....	1/75 "	"	36	" "	1/75 "	15,000	" "
9. 1-1,000....	1/80 "	"	1,260	" "	1/80 "	15,000	" "
10. 1-1,000....	1/90 "	"	1,190	" "	1/90 "	15,000	" "
11. 1-1,000....	1/100 "	"	6,300	Good growth.	1/100 "	15,000	" "
12. 1-1,000....	1/110 "	"	7,000	" "	1/110 "	15,000	" "
13. 1-1,000....	1/125 "	"	10,500	" "	1/125 "	15,000	" "
14. 1-1,000....	1/150 "	"	15,000	" "	1/150 "	15,000	" "
15. 1-1,000....	1/200 "	"	15,000	" "	1/200 "	15,000	" "

Control seeding, plated at once, 12,000; in four and one-half hours, 15,000.

A comparison of the figures in the fourth and seventh columns of this table indicate that activation was evident with a dilution of complement approximately three-fourths as high again as the dilution at which the fresh normal serum alone first failed to cause any diminution of the gonococci; and also that the dilution of complement best adapted for the detection of the immune bodies in this antigonococcic serum is at that point where the fresh normal serum alone begins to show a slight destructive action. As other like experiments confirmed this result, this optimum dilution was determined for the various normal rabbits and used in the activations.

The time element in the bacteriolysis. — It has been determined that three hours' incubation is sufficient for the bacteriolysis of cholera in experiments of this character, while with typhoid and paratyphoid bacilli it is advisable to carry out the plating after an incubation of about five hours. For the gonococcus the proper time of plating is dependent in a measure upon the amount of complement used in the reactivation of the immune serum; if so strong as to cause alone a marked destruction of the gonococci, the bactericidal action with the reactivated immune serum is complete within an hour; on the other hand, if present in so small amount as to have no bacteriolytic effect by itself, but sufficient to cause a certain amount of reactivation, the bactericidal action proceeds slowly and is still taking place at eight hours. If the complement is of such an amount as to cause optimum reactivation, but only slight destruction by itself, the plating may properly be carried out at some time after three hours. Four and one-half hours was chosen as giving a margin of safety.

Activated immune serums. — In the following tables (Tables VI. and VII.) are given the results of typical experiments with a reactivated gonococcic and a meningococcic immune serum. In the first column are placed the dilutions of the inactivated immune serum — each one-fourth the final dilution under the conditions of the experiment. In the second column is given the amount of fresh rabbit serum used for reactivation, and in the last two columns are described the appearance in twenty-four and also forty-eight hours of the various mixtures from which the platings were made. "Control complement" indicates the effect on the diplococci of the amount of fresh normal serum used in reactivation.

TABLE VI.

Bacteriolysis of gonococcus A by its specific serum.

Inactivated Immune Serum from Rabbit Inoculated with Gonococcus A, 8 times.	Complement Fresh Serum from Normal Rabbit No. 6.	Culture.	Colonies in 4½ Hours.	Tubes in 24 Hours.	Tubes in 48 Hours.
1. 1-5....	1/75 cc.	A.	10,000	Slight growth.	Good growth.
2. 1-10.....	" "	"	5,000	" "	" "
3. 1-25.....	" "	"	1,540	Very slight growth.	" "
4. 1-50.....	" "	"	296	" " "	" "
5. 1-100.....	" "	"	92	Slight growth.	" "
6. 1-500.....	" "	"	3	" "	" "
7. 1-1,000.....	" "	"	6	" "	" "
8. 1-2,000.....	" "	"	88	Good growth.	" "
9. 1-5,000.....	" "	"	280	" "	" "
10. 1-10,000.....	" "	"	700	" "	" "
11. 1-25,000.....	" "	"	4,200	" "	" "
12. 1-50,000.....	" "	"	5,000	" "	" "
13. 1-100,000.....	" "	"	6,300	" "	" "
14. Control Complement.....	1/75 cc.	A.	7,000	Good growth.	Good growth.
15. " Culture A Seeding.	—	"	15,000	" "	" "

Control seeding, plated at once, 12,000; media and sera, 0.

TABLE VII.

Bacteriolysis of a meningococcus culture by its specific serum.

Inactivated Immune Serum from Rabbit Inoculated with Meningococcus, 9 times.	Complement Fresh Serum from Normal Rabbit No. 6.	Culture.	Colonies in 5 Hours.	Tubes in 24 Hours.	Tubes in 48 Hours.
1. 1-5	1/20 cc.	Meningococcus.	9,440	Slight growth.	Fair growth.
2. 1-10	" "	"	4,200	" "	" "
3. 1-25	" "	"	1,260	" "	Good growth.
4. 1-100.....	" "	"	232	Very slight growth.	" "
5. 1-500	" "	"	172	" " "	" "
6. 1-1,000	" "	"	206	Fair growth.	" "
7. 1-2,000 ...	" "	"	490	" "	" "
8. 1-5,000	" "	"	1,890	Good growth.	" "
9. 1-10,000	" "	"	5,000	" "	" "
10. 1-25,000	" "	"	7,000	" "	" "
11. 1-50,000	" "	"	7,000	" "	" "
12. Control Complement	1/20 cc.	Meningococcus.	7,000	Good growth.	Good growth.
13. Control Culture Seeding ..	—	"	100,000	" "	" "
14. Meningococcus Serum 1-5 ...	—	"	22,000	" "	" "

Control seeding, plated at once, 12,000.

The bacterial counts in these tables, especially that with the gonococcus, parallel very closely those obtained in similar experiments with typhoid and paratyphoid. This particular gonococcic serum was rich in immune bodies, killing as it did an actual dilution of 1-40,000. As has been demonstrated by Neisser and Wechsberg³ and others, the extent of the prezone is dependent upon the degree in which the immune bodies are present in excess of the complementing elements. In the first tube, apparently, as noted in other instances, they were so far in excess as to abort in a measure the bactericidal power of the fresh normal serum alone. Where immune body and complement are in optimum ratio (as in tubes 5 and 6), there occur the greatest

destruction of gonococci. Although the tabulation of the colonies on the plates is very similar to that obtained with typhoid, the tubes from which the platings were made differ after twenty-four hours' incubation from those in a like experiment with this bacillus. Whereas, in the optimum killing zone with typhoid immune serum the tubes may be sterile in twenty-four hours, with gonococcus serum this does not occur in that a few cocci are always left which multiply slowly until in forty-eight hours the tubes are clouded with growth. It is possible that the incomplete killing is due to the necessary use of comparatively weak complement.

With the inactivated serum of a rabbit well immunized to the meningococcus (Table VII.) the killing zone was nearly as well marked and practically as extensive as with the gonococcus A. No experiments in vitro have been reported hitherto, which prove that meningococcic serum may be rich in bactericidal immune bodies. In comparing this table with the one dealing with gonococcus, it should be observed that a larger amount of complement was necessary for the reactivation of the meningococcic serum, and further that this particular strain of meningococcus multiplied much faster in the culture control tube, during five hours' incubation, than any strain of gonococcus which was tested.

The agglutination factor. — Soon after the publication of the Neisser-Wechsberg method, the criticism was advanced that agglutination might be a factor in the results obtained. This objection has been found invalid by Lipstein¹⁹ and others, but as the gonococcus is especially prone to spontaneous agglutination and is quickly clumped in comparatively high dilutions by immune serum, it seemed desirable to anticipate any criticism, based on this ground, of the soundness of these experiments. That agglutination, spontaneous or otherwise, is a negligible factor is proved by at least three considerations:

(a.) Fresh immune serum, at a dilution of 1-1,000, caused no decrease in the number of the gonococci, but when this same serum is reactivated, at this dilution the plates

were practically sterile and yet the degree of agglutination was necessarily the same in each instance.

(b.) In a previous article^{1b} it has been shown that strain A and strain G interagglutinate with their respective anti-serums in as high a dilution as 1-200 and yet reactivated A serum causes no decrease in the number of G colonies, nor G serum of A colonies.

(c.) Finally, in a parallel experiment with the same inactivated immune serum (Table VIII.), the typical "killing zone" occurred only in the series in which there was reactivation with fresh normal serum, hence the agglutination factor was of no significance.

TABLE VIII.

Experiment showing that agglutination is a negligible factor.

Inactivated Immune Serum from Rabbit inoculated with Gonococcus A 7 times.	Complement Fresh Serum from Normal Rabbit No. 8.	Culture.	Colonies in 4½ Hours.	Tubes in 24 Hours.	Same Immune Serum, but without Addition of Complement. Colonies in 4½ Hours.	Tubes in 24 Hours.
1. 1-2.....	—	A.	—	—	3,220	Very slight growth.
2. 1-5.....	1/100 cc.	"	3,640	No growth.	6,300	Slight growth.
3. 1-10....	" "	"	210	" "	7,800	" "
4. 1-25....	" "	"	15	Very slight growth.	13,700	Good growth.
5. 1-100...	" "	"	9	" " "	15,500	" "
6. 1-500...	" "	"	10	" " "	23,800	" "
7. 1-1,000..	" "	"	76	Fair growth.	18,500	" "
8. 1-2,000..	" "	"	288	Good growth.	—	—
9. 1-5,000..	" "	"	5,000	" "	16,800	Good growth.
10. 1-10,000.	" "	"	5,000	" "	21,800	" "
11. 1-25,000.	" "	"	13,000	" "	—	—
12. 1-50,000.	" "	"	11,000	" "	—	—
13. Control Complement.	1/100 cc.	A.	12,000	Good growth.	—	—
14. Control Culture A Seeding.	—	"	19,000	" "	22,400	Good growth.

Control seeding, plated at once, 10,000; same, 14,500; media and sera, 0.

Inhibitory action of inactivated immune serum alone. — Hüne¹² has described a weak but clear action of specific serums, without complement, in some experiments with typhoid and cholera and also in stronger dilutions with serums which were not specific. The latter, he thought, might be due in part to the phenol used as a preservative. From the figures in the sixth column of Table VIII. it will be seen that this inactivated serum immune to gonococcus A, without complement, caused some decrease in the number of colonies in a dilution as high as 1-10. Other inactivated serums, immune to strain H, have proved lytic without the addition of complement in a much more marked degree at this dilution, but only specifically. In these instances the action cannot be ascribed to a preservative as none was used. Several possible explanations of this lytic action suggest themselves, such as the presence of a complementing substance of a more stable nature, the bringing into play by the stronger dilutions of serum of an autolysate similar to that described by Flexner²⁰ for the meningococcus, or the presence of an inhibitory substance in the serum of the same nature as that produced in growing culture. At any rate it should be noted that the decrease in the count occurs alone in the prezone region and the phenomenon can play no part in the killing zone of the reactivated serum.

Inter-bactericidal action of various immune serums. — The question arises whether a reactivated anti-serum immune to one strain of the gonococcus will prove bactericidal for all other strains of this diplococcus; in other words, whether the immune bodies raised by various members of this group are homogeneous or heterogeneous. The same query is pertinent as regards the relationship from this standpoint of the gonococcus and the meningococcus. The following tables (IX, X., XI., XII., XIII.) elucidate these relationships as they have been found, by repeated tests, to exist between four strains of the gonococcus and between these and a single strain of the meningococcus. Each table contains the

results of the interaction of a single culture with each of the five anti-serums. Attention is directed to the amount of fresh normal serum (complement) employed with the several cultures. This is indicated in each case in conjunction with the "complement control" figures. It will be noted that it was necessary to vary the amount of complement. Guided by the experiments demonstrating that fresh normal serum in amount sufficient to cause in itself a slight destruction of the diplococci seeded was ample for the proper reactivation of the immune bodies, the dose of complement was so varied. As a result of this method of standardization, the delicate gonococcic cultures A and C received only about one-fourth as much complement as the more resistant strains G and H and also as this particular strain of the meningococcus. Whether or not this variability may be due to differences in the receptors of these strains, there is no decisive evidence. Attention is also directed to the fact that these strains of the gonococcus multiplied in the control tubes only slightly, if at all, in four or five hours.

TABLE IX.

Tests with gonococcus culture A against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Immune to Gonococcus A. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus C. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus G. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus H. Colonies in 4½ Hours.	Result with Serum Immune to Meningococcus. Colonies in 4½ Hours.
1. 1-5.....	10,000	25,000	11,500	15,000	6,000
2. 1-10.....	5,000	9,000	—	—	—
3. 1-25.....	1,540	5,000	10,500	15,000	4,000
4. 1-100.....	92	200	12,500	15,000	9,500
5. 1-500.....	3	112	14,000	15,000	22,000
6. 1-1,000.....	6	328	11,500	15,000	7,500
7. 1-2,000.....	88	—	—	—	—
8. 1-5,000.....	280	14,000	14,000	15,000	8,000
9. 1-10,000...	700	14,000	—	—	—
10. 1-25,000...	4,200	25,000	—	—	—
11. 1-100,000...	6,300	—	—	—	—
Control with Complement.	Normal Rabbit No. 6, Serum 1/75 cc. 7,000	Normal Rabbit No. 2, Serum 1/100 cc. 29,000	Normal Rabbit No. 3, Serum 1/100 cc. 12,500	Normal Rabbit No. 3, Serum 1/125 cc. 15,000	Normal Rabbit No. 8, Serum 1/100 cc. 5,500
Control Culture Seeding.	15,000	30,000	18,000	16,000	10,000
Control Seeding, plated at once.	12,000	25,000	16,000	14,000	8,000

TABLE X.

Tests with gonococcus culture C against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Immune to Gonococcus A. Colonies in $4\frac{1}{2}$ Hours.	Result with Serum Immune to Gonococcus C. Colonies in $4\frac{1}{2}$ Hours.	Result with Serum Immune to Gonococcus G. Colonies in $4\frac{1}{2}$ Hours.	Result with Serum Immune to Gonococcus H. Colonies in $4\frac{1}{2}$ Hours.	Result with Serum Immune to Meningococcus. Colonies in $4\frac{1}{2}$ Hours.
1. 1-5.....	4,200	24,000	4,200	50,000	12,000
2. 1-10.....	1,050	19,000	—	—	—
3. 1-25.....	152	15,500	5,000	50,000	12,000
4. 1-100.....	140	315	5,000	50,000	12,000
5. 1-500.....	3,250	215	5,500	50,000	12,000
6. 1-1,000.....	9,800	175	7,000	50,000	12,000
7. 1-2,000.....	18,000	—	—	—	—
8. 1-5,000....	19,000	560	8,400	50,000	12,000
9. 1-10,000....	19,000	6,000	—	—	—
10. 1-25,000....	19,000	17,000	—	—	—
11. 1-50,000...	19,000	15,000	—	—	—
Control with Complement.	Normal Rabbit No. 6, Serum 1/75 cc. 15,000	Normal Rabbit No. 2, Serum 1/100 cc. 26,000	Normal Rabbit No. 3, Serum 1/100 cc. 6,000	Normal Rabbit No. 3, Serum 1/125 cc. 45,000	Normal Rabbit No. 3, Serum 1/125 cc. 12,000
Control Culture Seeding.	25,000	30,000	5,500	50,000	18,000
Control Seeding, plated at once.	24,000	28,000	6,500	50,000	18,000

TABLE XI.

Tests with gonococcus culture G against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Immune to Gonococcus A. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus C. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus G. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus H. Colonies in 4½ Hours.	Result with Serum Immune to Meningococcus. Colonies in 4½ Hours.
1. 1-5.....	14,500	4,500	0	16	5,000
2. 1-10.....	—	—	0	30	—
3. 1-25.....	8,400	6,000	0	2,800	5,700
4. 1-100.....	12,000	4,500	1	17,000	5,000
5. 1-500.....	12,000	6,500	350	18,000	5,000
6. 1-1,000.....	14,000	7,800	840	18,000	4,480
7. 1-2,000.....	—	—	1,680	14,000	5,000
8. 1-5,000.....	—	7,500	2,170	18,000	—
9. 1-10,000.....	—	—	3,300	18,000	—
10. 1-25,000.....	—	—	3,200	18,000	—
Control with Complement.	Normal Rabbit No. 1, Serum 1/30 cc. 14,000	Normal Rabbit No. 6, Serum 1/20 cc. 5,500	Normal Rabbit No. 4, Serum 1/20 cc. 3,500	Normal Rabbit No. 4, Serum 1/20 cc. 18,000	Normal Rabbit No. 7, Serum 1/20 cc. 5,000
Control Culture Seeding.	28,000	9,000	10,000	19,000	6,000
Control Seeding, plated at once.	20,000	7,500	7,000	—	5,000

TABLE XII.

Tests with gonococcus H against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Im- mune to Gono- coccus A. Colonies in 4½ Hours.	Result with Serum Im- mune to Gono- coccus C. Colonies in 4½ Hours.	Result with Serum Im- mune to Gono- coccus G. Colonies in 4½ Hours.	Result with Serum Im- mune to Gono- coccus H. Colonies in 4½ Hours.	Result with Serum Im- mune to Men- ingococcus. Colonies in 4½ Hours.
1. 1-5	2,900	3,500	1	280	5,700
2. 1-10.....	—	—	4	106	—
3. 1-25.....	2,170	2,800	15	102	5,000
4. 1-100.....	3,220	1,640	280	210	5,000
5. 1-500.....	2,870	2,800	2,240	1,300	5,000
6. 1-1,000	2,730	4,000	4,200	3,850	5,000
7. 1-2,000	—	—	5,320	8,500	—
8. 1-5,000	2,800	—	8,500	10,500	5,000
9. 1-10,000 ...	—	—	8,680	11,500	—
10. 1-25,000....	—	—	9,200	14,000	—
Control with Complement.	Normal Rabbit No. 6, Serum 1/25 cc. 3,500	Normal Rabbit No. 7, Serum 1/25 cc. 4,900	Normal Rabbit No. 6, Serum 1/30 cc. 8,500	Normal Rabbit No. 4, Serum 1/25 cc. 14,000	Normal Rabbit No. 7, Serum 1/35 cc. 5,000
Control Culture Seeding.	5,000	8,000	11,000	21,000	6,720
Control Seeding, plated at once.	4,200	7,000	9,000	18,000	11,000

TABLE XIII.

Tests with meningococcus against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Immune to Meningococcus. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus A. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus C. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus G. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus H. Colonies in 4½ Hours.
1. 1-5.....	13,000	750	700	8,060	10,000
2. 1-10.....	12,000	—	—	—	—
3. 1-25.....	4,000	5,600	2,800	18,000	17,000
4. 1-100.....	1,400	12,000	5,000	30,000	50,000
5. 1-500.....	210	15,000	5,500	100,000	50,000
6. 1-1,000.....	220	15,000	5,600	100,000	50,000
7. 1-2,000.....	420	—	—	—	—
8. 1-5,000.....	1,950	15,000	7,000	100,000	50,000
9. 1-10,000.....	2,700	—	—	—	—
10. 1-25,000.....	7,500	—	—	—	—
Control with Complement.	Normal Rabbit No. 8, Serum 1/30 cc. 11,000	Normal Rabbit No. 3, Serum 1/40 cc. 14,000	Normal Rabbit No. 6, Serum 1/20 cc. 7,000	Normal Rabbit No. 3, Serum 1/30 cc. 33,000	Normal Rabbit No. 3, Serum 1/30 cc. 30,000
Control Culture Seeding.	30,000	50,000	35,000	100,000	100,000
Control Seeding, plated at once.	4,300	5,500	11,000	14,000	10,000

The essential point brought out in these experiments lies in the demonstration that the gonococcic strains A and C produce, in rabbits, immune bodies which cause mutual bacteriolysis, but none which react with strains G and H; and further, that G and H raise such anti-bodies reacting on one another to some extent (the action of H serum on culture G is slight), but none for strains A and C. Again, that all four of these antigenococcic serums were slightly destructive for the meningococcic culture. Finally, that this strain of the meningococcus produced an anti-serum strongly bactericidal for itself, but in no degree on any of the four gonococcic strains. It is, also, to be observed that

cultures G and H stimulated apparently the production of fewer immune bodies than A, C, or the meningococcus, as is indicated by the comparatively low specific bactericidal titer of their anti-serums and the complete absence of prezones.

In a previous article ¹⁶ on the "fixation of complement" with serums immune to these same strains, it was demonstrated that cultures A and C reacted alike, as did also to a certain extent cultures G and H, but that there was no interaction between these two pairs. This result is interesting in that it indicates, at least, a parallelism in specificity between the "fixation of complement" and these bacteriolytic experiments. Whether, however, we may say that these two processes are identical as regards the bacterial amboceptors concerned is left for further experimentation and will be discussed in another place.

The gonococcus group. — Culturally and morphologically the gonococcus group is apparently homogeneous. Such differences as may appear between various strains in their aptitude to adapt themselves to an unfavorable environment (culturally) are of slight significance, as these are variable and merely matters of degree. As regards their reaction to strains there seems to be a uniformity within the group to the smallest detail. Their enzymatic activities are feeble and probably uniform. In fact it is apparently only within the field of these more delicate serum reactions that heterogeneity may be observed. First, by a study of agglutination ¹⁵ with specific serums radical differences were evident between certain strains; a diversity, which was again manifest in the delicate "fixation of complement" test, ¹⁶ and finally confirmed by these bacteriolytic experiments.

Although agglutination seemed to disclose a surprising amount of variation within the group, certain cultures reacted practically alike and fell within two or more sub-groups. In the subsequent experiments my aim has been to determine how radically as regards other serum reactions two of these sub-groups differed from one another: the one sub-group represented by cultures A, B, and C, or let us say Type I.;

and the other by cultures G, H and I, or Type II. These differences, as determined, may be summarized as follows:

(a.) These two types raise certain agglutinins which are common, but their specific agglutinins are entirely distinct.

(b.) In the fixation of complement the cultures grouped under Type I. manifested a practical identity as regards antigen and anti-body; and the cultures of Type II. showed a similar uniformity among themselves. Yet there was little or no interaction between the members of these two sub-groups, indicating, thereby, the existence of a radical difference between the antigens and the anti-bodies derived from and with these two types.

(c.) Again, the cultures of Type I. are decidedly more sensitive to the bacteriolytic action of fresh normal rabbit serum than those of Type II.

(d.) Finally, the bactericidal immune bodies raised by the members of Type I. are inter-active among themselves, but are inactive with those of Type II.; and the reverse is true as regards the Type II. cultures.

We find, then, certain strains of the gonococcus which are alike in antigen, in the anti-bodies which they call forth, and in their receptor apparatus, but also others which are totally different. Is it necessary to count such subtle differences important or may they, from a practical standpoint, be disregarded? Is such heterogeneity of fundamental significance in the serum-therapy of gonococcic or other infections? Unfortunately we are still almost entirely in the dark as to the mode of action of bactericidal serums, when introduced into the body. That the bactericidal immune bodies specifically active in vitro against certain micro-organisms are of no significance in bacteriolysis in vivo has not been proved,* and until such proof is forthcoming it is only reasonable to suppose that these immune bodies may be active agents in some curative serums. Elsewhere

* Töpfer and Jaffé,* among others, hold the view that the bacteriolytic process in vitro and in the animal body are not identical. They found during sickness with typhoid that in vitro test high and the Pfeiffer lower, but during convalescence Pfeiffer high, protecting strongly, and in vitro feeble.

certain evidence has been given by the writer² which indicates that this may be the case with antigonococcic serum. Granting for the time being that bactericidal immune bodies, as revealed in test-tube experiments, are of significance in the curative process, these results are of practical importance in that they show:

(a.) That a serum produced with one strain of the gonococcus may be ineffective if the patient harbors a strain of this diplococcus belonging in a different sub-group. This being so it is desirable to determine the number of these sub-groups and to employ as many of them as possible in the preparation of a therapeutic serum. Shiga's²¹ decidedly efficient anti-dysenteric serum now contains antibodies for all of the five main groups of *B. dysenteriae*.

(b.) That certain strains of the gonococcus are very readily destroyed by fresh normal serums and by their specific anti-serums, while others succumb less readily to such lytic agents. It is possible that this fact is of some weight in determining the transitory character of certain cases of gonorrheal infection as compared with the chronic nature of others.

CONCLUSIONS.

1. Certain strains of gonococci are very sensitive to the bactericidal action of fresh normal rabbit serum, while others are decidedly more resistant.
2. Bactericidal immune bodies are readily produced in rabbits by inoculation with the gonococcus and also by the meningococcus.
3. A serum immune to one strain of the gonococcus may be entirely inactive in vitro against another strain.
4. All of the inactivated antigonococcic serums (four) tested in these experiments were slightly bacteriolytic for a certain strain of meningococcus. An antimeningococcic serum contained no bactericidal immune bodies for four strains of the gonococcus.
5. These experiments indicate that there is a parallelism

in the specificity of the results obtained by the "fixation of complement" method and in vitro bactericidal tests. They likewise confirm the conclusion, previously expressed, that the gonococcus group is heterogeneous.

REFERENCES.

1. Torrey. * Journ. Am. Med. Asso., 1907, xliv, 918.
2. Torrey. This Journal, 1908, xviii, 347.
3. Neisser and Wechsberg. Münchener Med. Woch., 1901, No. 18.
4. Neisser. Gesammelte Arb. zur. Immunitats. von Ehrlich, 1904, 493.
5. Stern and Krote. Berl. klin. Woch., 1904, xli, 213.
6. Krote and Steinberg. Deutsches Archiv. f. klin. Med., 1905, lxxxii, 321.
7. Laubenheimer. Zeitschr. f. klin. Med., 1905, lvi, 170.
8. Töpfer and Jaffé. Zeitschr. f. Hygiene, 1906, lii, 393.
9. Neufeld and Hüne. Arb. a. d. Gesundheitsamte, 1907, xxv, 16.
10. Wright. Lancet, 1901, 609.
11. Shiga. Berl. klin. Woch., 1904, xli, 79.
12. Hüne. Arb. a. d. Gesundheitsamte, 1907, xxvi, 196.
13. Buxton. This Journal, 1905, xlii, 305.
14. Cohn. Zeitschr. f. Hygiene, 1903, xlv, 61.
15. Torrey. This Journal, 1907, xvi, 329.
16. Teague and Torrey. This Journal, 1907, xvii, 223.
17. Buxton. This Journal, 1905, xlii, 431.
18. von Nadoleczny. Archiv. f. Hygiene, 1900, xxxvii, 277.
19. Lipstein. Gesammelte Arb. zur. Immunitats. von Ehrlich, 1904.
20. Flexner. Journ. Exper. Med., 1907, ix, No. 2.
21. Shiga. Zeitschr. f. Hygiene, 1908, lx, 75.

METABOLISM IN TYPHOID FEVER.*

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I shall not attempt to review the many-sided subject of metabolism in typhoid fever, but shall devote the time to a brief discussion of one phase of the subject, which is of great importance, as well as of interest, both to the clinician and to the pathologic physiologist.

During the course of the disease a typhoid patient loses from ten to sixty pounds, or even more, of his body tissue. This loss is divided among water, subcutaneous fat and protein from the body fluids or cells. The loss of water we may leave out of consideration because we know neither its amount or significance. Since the observation of Leyden¹ in 1869 it has been believed that there is a retention rather than a loss of water from the body in fever. This belief is supported by the decreased quantity of urine and by the supposed decrease in the evaporation from the skin; but few or no accurate data on this question are available, and no positive statements concerning it can be made.

The burning of body fat may, in the absence of carbohydrates, and perhaps also other conditions, lead to varying degrees of acidosis, with an abstraction of alkalis from the tissues, and possibly in other ways may be of distinct harm to the patient. This is borne out by the clinical observation that fat individuals are comparatively poor subjects for typhoid fever, or for that matter, for any infectious fever or for surgical operations. We have, however, as yet no good reason for believing that the metabolism of fat in fever is in anv

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1. Arch. f. klin. Med., 1869, v, 366.

way different qualitatively from that in other conditions of undernutrition; and it seems to me probable that the excretion of the acetone bodies occasionally recorded in typhoid fever is merely the result of a burning of body fat in the absence of sufficient carbohydrates—the frequently observed starvation acidosis. If this is true, acetone, diacetic and beta-oxybutyric acids will not be excreted by the typhoid patient who receives an abundance of carbohydrates. Aside from the production of organic acids in the burning of body fats, the mere loss of fat is probably of no great consequence.

In addition to the loss of body fat there is a great loss of body protein in typhoid fever, and this is what I shall speak of in some detail. Many data are to be found in the literature as examples of the amount of this loss of protein. In a case reported by Leyden and Klemperer² there was a loss of 109 gm. of nitrogen, the equivalent of 3.2 kilos or seven pounds of pure muscle tissue in twelve days. In a case of Frederick Müller³ there was a loss of 86.4 gm. of nitrogen, the equivalent of 2.5 kilos or five and one-half pounds of muscle tissue in eight days. These are not very unusual figures; a loss of the equivalent of even one and one-half pounds of muscle tissue in a single day is not very rare. There are many reasons for believing that this febrile loss of protein from the body is a serious and dangerous proceeding. The emaciation, muscular weakness and decreased resistance, and the long convalescence are certainly in part the results of the loss of body protein. The amount of protein lost appears to bear a close relation to the severity of the disease, and Ewing believes that the pathologic processes concerned in the metabolism of this body protein take a prominent part in determining the patient's condition. Ewing's⁴ idea is that many of the phenomena of typhoid fever, especially in the severe and fatal cases, are due to an autointoxication resulting from the "burning of thirty pounds of body tissue in three weeks"—and not directly to the endotoxins of typhoid bacilli. This idea is to some extent supported by the fact that the so-called nitrogen partition of the urine in severe or fatal cases is decidedly

2. Von Leyden: *Handbuch der Ernährungstherapie*, 1904, II.

3. *Cong. f. inn. Med.*, 1902, p. 192.

4. *Proc. Path. Soc. Philadelphia*, 1905; paper before New York Acad. Med., *New York Med. Rec.*, 1907, p. 537.

abnormal, and similar to those found by Wolf, Ewing and others in toxemia of pregnancy. Furthermore there are severe so-called toxic cases of typhoid fever which terminate with acute yellow atrophy of the liver, a condition which appears to be closely associated with a particular type of faulty protein metabolism. But aside from the possibility of its creating an autointoxication, the consumption and loss of body protein must be of great harm to the patient, both during the disease and during convalescence.

The causes for this loss of body protein are apparently three in number.

The first is partial starvation. An individual is obviously undernourished unless he absorbs from the digestive tract food of sufficient caloric value to equal the energy expended. Except by very difficult and accurate measurements it is impossible to know the amount of energy being expended by a particular patient, and few such measurements have been made on fever patients; but we may readily calculate average figures which are satisfactory for practical purposes.

At ordinary rest the heat and other energy expended by a normal individual receiving sufficient food is about thirty-three calories per kilo body weight. In fever there is an increased heat production, with an average, according to Krehl, of 20 to 30 per cent. Twenty-five per cent. added to the thirty-three calories gives about forty calories, or, for a patient weighing seventy kilograms, or 150 pounds, 2,800 calories. This represents the minimum amount of energy which the average typhoid patient is expending in each twenty-four hours. If he does not receive food equivalent to this amount of energy he merely draws on his body tissues to make up for the deficit. Few or no typhoid patients receive enough food to maintain an equilibrium, and they consequently burn up for fuel varying amounts of their body fat and protein.

Any physician can readily calculate the probable deficit with the diets he uses. The deficit in calories is usually about 50 per cent., and this, according to von Noorden's figures,⁵ may be responsible for loss of 2 to 3 gm. of body nitrogen per day.

5. *Handbuch der Pathologie des Stoffwechsels*, 1906, 1, 497.

The two other causes for the febrile loss of body protein are the pyrexia and the action of the bacterial toxins. From the experiments of Linser and Schmidt,⁶ Fritz Voit⁷ and Schleich,⁸ we know that artificially raising the body temperature causes an increase in protein metabolism. The pyrexia itself is therefore one of the causes for the loss of body protein; but the loss due to this cause can be prevented, just as the loss due to partial starvation, by the intake of sufficient food.

The third factor is the so-called "toxic" destruction of body protein from the poisonous action of the bacterial toxins on the body protein. This last factor is still open to discussion, but I am inclined to the belief that some such action does take part. It is, however, at present impossible to discuss the toxic destruction apart from the result of the pyrexia, because we can not distinguish between the two when both are present, as they usually are in typhoid. The question which is of importance for the practical treatment of typhoid is, can the combined effect of the pyrexia and the bacterial toxins be prevented from causing a loss of body-protein? There are already some encouraging answers to this question. May,⁹ working with rabbits infected with pig erysipelas, was able to decrease the febrile loss of body protein by carbohydrates; though May later doubted the correctness of his first interpretation. Puritz¹⁰ worked on human typhoid in St. Petersburg, and his results led him to favor a liberal diet in this disease. His diets, however, were rich in protein and of only moderate caloric value, and were therefore, I believe, not well adapted for his purpose, which was to retard the loss of protein. Weber,¹¹ working with a sheep inoculated with an extract of glanders bacilli, was able wholly to prevent any febrile loss of body protein by giving the animal a liberal diet containing much carbohydrate. Leyden and Klemperer, on the other hand, conclude from their experiments on typhoid and pneumonia that, while

6. Arch. f. klin. Med., lxxix, 514.

7. Sitzungsab. d. Gesellsch. f. Morphol. u. Physiol. in München, 1895, No. 2.

8. Arch. f. exp. Pathol. u. Pharmacol., iv, 82.

9. Ztschr. f. Biol., 1894, xxx, 1.

10. Virchows Arch. f. Path. Anat., 1893, cxxxi, 327.

11. Arch. f. exper. Path., xlvii, 10.

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a liberal diet is desirable, it is not possible to prevent the febrile loss of protein; this view is generally accepted.

Last summer Dr. Warren Coleman and I undertook in Bellevue Hospital, New York, a further study of the extent to which the loss of body protein in typhoid fever might be retarded by dietetic means.¹² Our results were on the whole very encouraging, in that we were able to diminish the loss of body nitrogen to a comparatively small amount during the fastigial temperature and were able to make patients gain body protein during the steep curve period of the disease.

I need not here go fully into the reasons underlying our choice of diet, which is the opposite of that used by Puritz, and indeed the opposite, in some particulars, of the diets at present used in typhoid fever by most physicians. Our principle has been to have the diet contain a moderate amount of protein and the largest possible amount of carbohydrate. The reasons for choosing carbohydrates as the basis of the diets were briefly as follows: Carbohydrates are the strongest spacers of body protein in health, according to Voit, Lusk, Folin and others; and the experiments of May, Weber and Linser and Schmidt indicate that this sparing action takes place in fever as it does in health.

A large amount of fat is objectionable because of its tendency toward digestive disturbances and diarrhea; and the protein should be kept at the minimum effective amount because of its action in increasing the heat production (Rubner) and because of the large amount of work it throws on the intestine and the kidneys. The results from one of our cases are given in the accompanying chart. Samples of the diets used are given in the table.

Our results with other cases show essentially the same thing, although in some cases there was no gain, but a continued slight loss of one gram or so per day. On the high caloric diets, however, the patients were almost always in a condition not far from nitrogen equilibrium.

Our results show, we believe, that the febrile loss of

12. We are indebted to Dr. Armstrong, medical superintendent of Bellevue Hospital, for placing the facilities of the hospital at our disposal. The experiments will be published elsewhere in detail.

body protein, including the result of the three factors, undernutrition, pyrexia and the action of toxins, may be retarded and even wholly prevented, or compensated for. But the results show likewise how difficult this is

TABLE OF COMPOSITION OF FOOD DURING EXPERIMENT
SHOWN IN CHART.

The numbers at the left refer to corresponding numbers on the "nitrogen balance" curve in chart.

	Gm.	Calories.	Per Cent. Total Calories.	Calories Per Kg. Body Wt.
1. { Protein.....	60	245	7.8	
Fat.....	80	745	23.7	
Carbohydrate.....	525	2150	68.5	
		3140		58.
2. { Protein.....	56	230	6.2	
Fat.....	78	725	19.5	
Carbohydrate.....	670	2750	74.3	
		3705		69.
3. { Protein.....	53	217	8.7	
Fat.....	63	585	23.6	
Carbohydrate.....	410	1680	67.7	
		2482		46.
4. { Protein.....	75.5	310	8.2	
Fat.....	120.4	1120	29.8	
Carbohydrate.....	569	2330	62.0	
		3760		70.
5. { Protein.....	16.2	66	2.0	
Fat.....	108	1004	30.8	
Carbohydrate.....	547	2240	67.7	
		3310		62.
6. { Protein.....	84	345	20.0	
Fat.....	96	898	51.7	
Carbohydrate.....	120	490	28.3	
		1728		32.
7. { Protein.....	85	349	8.8	
Fat.....	122	1234	28.7	
Carbohydrate.....	583	2390	62.5	
Alcohol.....		80		
		3953		73.

to accomplish. It was only when we gave sixty to seventy or even eighty calories per kilogram—between 3,000 and 4,000 calories—that the greatest sparing was observed.

The important point is, however, that it is possible to give typhoid patients such liberal diets without, so far

as our experience shows, producing any harmful results, but, we believe, with decided benefit.

Having learned that it is possible to retard the febrile loss of body protein, we have still to decide whether it is desirable to do so in typhoid fever.

There are many objections, some apparent and others real, and many formidable difficulties; but I know of no reason why we should not attempt to do all in this direction that the circumstances will allow. Among the possible objections to liberal feeding in typhoid is the commonly supposed digestive limitation of these patients. The digestion in typhoid fever undoubtedly has its limitations, but they are not such as to prevent the proper absorption of amply sufficient food if given in the proper form. The experiments of von Hoesslin,¹³ Leyden and Klemperer,¹² Puritz,¹⁰ Folin¹⁴ and others show very positively that the average typhoid patient absorbs food from his intestine almost as completely as does the healthy individual. Only in the severest cases is the absorption very materially decreased. In the average case without profuse diarrhea the digestion of protein, fat and carbohydrate is within 10 or 15 per cent. of the normal. Folin,¹⁴ in his recent work on typhoid, has directly determined the degree of absorption of carbohydrates and has found it practically normal.

What seems to me an objection to liberal feeding in typhoid fever is the effect of the protein in increasing the heat production. Rubner¹⁵ has shown how a strict protein diet in a dog may increase the heat production more than 50 per cent. The effect in a human patient could never be so great, but with the decreased heat loss in fever it is quite possible that this factor may be of considerable importance. This objection does not hold against the diets Coleman and I have used, but may apply to the high protein diets used by others. Carbohydrates have only a very slight effect in increasing heat production.

The objection has been raised to the use of large quantities of carbohydrates, from the fear of fermentation

13. Virchows Arch. f. Path. Anat., 1882, lxxxix, 317.

14. Unpublished experiments.

15. Rubner: Die Gesetze des Energieverbrauchs, 1902.

and tympanites. I can only say that we have had no such experiences with the use of milk sugar or starch.

The greatest practical difficulty that we have so far encountered in this work is the choice of food products. After trying or considering various carbohydrates we have used milk sugar almost wholly. This has the advantage of fermenting only with difficulty and of being less sweet than cane sugar and much more soluble than any form of starch. Milk, diluted cream, and eggs have been used to furnish protein and fat. Cocoa, lemon juice, tea, coffee and other things have been used as flavoring agents and as vehicles for milk sugar.

The patients did not object to our diets more than to milk alone, but some persuasion was frequently necessary to get them to take the amounts of food found to be necessary for our purposes.

The full advantages as well as the possible objections to our dietetic plan have still to be demonstrated; but there are a few reasons in favor of such liberal nourishment for typhoid patients which are worthy of consideration at this time.

As I pointed out earlier, the average typhoid patient receives at present 50 per cent. or less of his energy requirement. This is half starvation; and we know that starvation is harmful even in health, in that it leads to weakness and to an increased susceptibility to many infectious diseases. If starvation is harmful in health why should it be beneficial in typhoid fever? During the course of typhoid fever great demands are constantly being made on the defensive power of the organism; and it certainly does not seem probable that the patient will be as well prepared to meet those demands when in a starved or half-starved conditions as when he is being supplied with sufficient energy in the form of food.

It is a common laboratory observation that strong, robust, well-fed animals develop the strongest artificial immunity; and it seems fair to believe that we are assisting the patient to acquire his immunity to typhoid fever by keeping his nutrition at the highest possible level. Is it not possible that one of the factors determining the outcome of the disease may be the state of nutrition in which the body cells are maintained? Retarding the loss of body protein should leave the patient

at the end of the fever in better physical condition and so make possible a shorter convalescence.

This sort of reasoning is, however, largely speculative; and long experience alone can tell the true value of such treatment. We can merely say at present that it is possible by the means already outlined to retard and even to prevent the febrile loss of body protein.

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A METHOD FOR THE QUANTITATIVE DETERMINATION OF β -OXYBUTYRIC ACID IN URINE.

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We have at present for the determination of β -oxybutyric acid, no method which is not open to serious criticism, either on the point of accuracy or on account of time, labor and chemicals involved. The method of separately determining the inorganic acids and bases of the urine, and so calculating the amount of organic acid present, was first used for this purpose by Stadelmann¹ who adopted it from Gähtgens,² and from its use Stadelmann was led to the discovery of the organic acid, thus verifying the statement made three years earlier by Hallervorden that the high ammonia content of diabetic urines is due to the presence of an organic acid.³

This procedure has since been used in the study of β -oxybutyric acid in relation to acidosis by Magnus-Levy⁴ and others. The method is very laborious, necessarily inaccurate, on account of the many operations required, and still more important, it does not determine the amount of β -oxybutyric acid, but merely the amount of total organic acidity. And for this latter purpose the simpler and more accurate method proposed by Folin⁵ is to

¹ Stadelmann: *Arch. f. exp. Path. u. Pharm.*, xvii, p. 419, 1883.

² Gähtgens: *Zeitschr. f. physiol. Chem.*, iv, p. 36, 1880.

³ Hallervorden (*Arch. f. exp. Path. u. Pharm.*, xii, p. 237) in 1880 confirmed the finding of Boussingault of high ammonia excretion in diabetes, and suggested that it was caused by the excretion of an organic acid (lactic or glycuronic acids). Stadelmann in 1883 confirmed Hallervorden and isolated crotonic acid. In 1884 Külz, *Zeitschr. f. Biol.*, xx, p. 165; *Arch. f. exp. Path. u. Pharm.*, xviii, p. 290, and Minkowski, *Arch. f. exp. Path. u. Pharm.*, xviii, pp. 35 and 147, independently showed the acid to be β -oxybutyric.

⁴ Magnus-Levy: *Arch. f. exp. Path. u. Pharm.*, xlii, p. 149, 1899.

⁵ Folin: *Amer. Journ. of Physiol.*, ix, p. 265, 1903.

be preferred. With one exception, the other methods for the determination of β -oxybutyric acid are based upon the optical activity of the lævorotary acid or its salts.

The rotation of the fermented urine is read in the polariscope (Külz) with or without preliminary treatment with basic lead acetate; or the acid is extracted by ether from the evaporated urine, and the rotation of an aqueous solution of the residue from the ether is determined, and the amount of oxybutyric acid calculated.

Direct reading of the fermented urine is easy enough but the results are worthless because of, first, the great percentage error in reading dilute solutions of the acid or its salts;¹ second, the probable presence of other optically active substances in urine even after fermentation; and third, if basic lead acetate be used, the action of this substance in increasing the lævorotation of salts of the acid (Magnus-Levy).² Extraction of the acid by ether, and subsequent polarization of the aqueous solution of the residue from the ether was apparently first used by Wolpe³ in 1886. Without altering the principle of the method, it has been very materially improved by Magnus-Levy, Bergell,⁴ and most recently by Black. With the use of the latter's modifications which consist in dehydrating the evaporated urine with plaster of paris, and the use of an improved continuous ether-extraction apparatus,⁵ the method is fairly quick and the results may be fairly satisfactory. The principle of the method is still however open to the objection of difficulty of complete extraction, and that other optically active substances may be extracted from the urine,

¹ The specific rotation of the free acid is -24.12° and of the sodium salt -14.35° (Magnus-Levy: *Arch. f. exp. Path. u. Pharm.*, xlv, p. 393 and 397, 1901). The specific rotation is different for the salts with different bases. Magnus-Levy points out that an error of 0.10° in reading the polariscope would amount to about 15 grams in 5 liters of urine (*ibid.*, xlii, p. 170, 1899). An error of 0.10° in reading a 2 per cent solution of β -oxybutyric acid (which is frequently obtained from urines containing only a little of this acid) would amount to more than 10 per cent of the total.

² Magnus-Levy: *Arch. f. exp. Path. u. Pharm.*, xlv, p. 393, 1901.

³ Wolpe: *Arch. f. exp. Path. u. Pharm.*, xxi, p. 138.

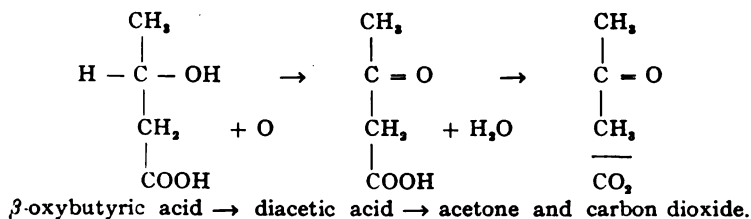
⁴ Bergell: *Zeitschr. f. physiol. Chem.*, xxxiii, p. 310, 1901.

⁵ Bergell used anhydrous copper sulphate and extracted in a Soxhlet apparatus.

and either increase or decrease the laevorotation of the final solution.

The method proposed by Darmstädter¹ is, from its author's description, very easy and exceedingly accurate; though in my hands it has not been successful. After evaporating the urine made alkaline with sodium carbonate Darmstädter distills it with a constant concentration of 50 per cent sulphuric acid thus converting the β -oxybutyric acid into crotonic acid which distills over. The distillate of 300 to 400 cc. is extracted *three* times with ether which, he claims, removes all of the crotonic acid. The ether is distilled and the residue, containing the crotonic acid, after being heated on a sand-bath to remove the volatile acids, is dissolved in water and titrated with alkali. Darmstädter in this way obtained results from 99.36 per cent to 99.70 per cent of the amount of synthetic β -oxybutyric acid added to urine. Unfortunately he does not state just how he determined so accurately the amount of oxybutyric acid used in his experiments. Of the various objections to this method as its author describes it, the most obvious is perhaps the difficulty in completely extracting the crotonic acid from 300 cc. of liquid, by two or three portions of ether. The method has not apparently been received with favor.

It occurred to me that it might be possible to utilize as the basis for a new method a property of β -oxybutyric acid long ago mentioned by Minkowski—its oxidation with the formation of acetone and carbon dioxide. I hoped that the reaction might, under certain conditions, proceed after the following well known scheme.



Distilling with sulphuric acid and potassium bichromate, under the conditions to be described, it is easily possible to obtain from β -oxybutyric acid the theoretical amount of acetone, the quantity

¹ Darmstädter: *Zeitschr. f. physiol. Chem.*, xxxvii, p. 355, 1903.

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of which may be accurately determined by means of standard iodine and thiosulphate solutions.

The conditions necessary for a maximum yield of acetone concern the concentrations of sulphuric acid, and of bichromate. Too little sulphuric acid, even with an excess of bichromate liberates the acetone very slowly and perhaps incompletely; while a very great excess of sulphuric acid decomposes β -oxybutyric acid with the formation of crotonic acid. The optimum concentration appears to be between 3 per cent and 7 per cent sulphuric acid. The following experiments show the effect of varying amounts of sulphuric acid upon the speed with which the acetone is formed.

The determinations were made in a fairly pure solution of inactive β -oxybutyric acid, made by the reduction of acetacetic ester by sodium amalgam (Wislicenus).¹ About 90 per cent of the titrated acidity of the solution was due to oxybutyric acid (from the determination of acetone under conditions giving the correct results).

The volume of each distillate was about 300 cc.; more water was added to the distilling flask before each subsequent distillation or the volume was kept constant at about 500 cc. by means of water from a dropping funnel.

The acetone in the distillates was determined as usual with standard (103.5 per cent $\frac{N}{10}$) thiousulphate and iodine solutions, of which 1 cc. = 1 mg. acetone. The results are given in milligrams of acetone.

With 1.0 gram potassium bichromate:

I.	1 cc. H_2SO_4	1st	dist.	16.0	mg. acetone.*
		2d	"	18.8	"
		3d	"	7.0	"
		4th	"	2.4	"
				44.8	"
II.	2 cc. H_2SO_4	1st	dist.	21.5	"
		2d	"	19.0	"
		3d	"	6.2	"
		4th	"	1.7	"
				48.4	"
III.	5 cc. H_2SO_4	1st	dist.	39.1	"
		2d	"	10.6	"
				49.7	"

¹ Wislicenus: *Ann. d. Chem.*, cxlix, p. 205, 1869.

IV.	20 cc. H_2SO_4	1st dist.	47.0	mg. acetone.
		2d "	0.0	"
			<hr/> 47.0	"
V.	50 cc. H_2SO_4	1st dist.	40.6	"
		2d "	0.0	"
			<hr/> 40.6	"
VI.	50 cc. H_2SO_4	1st dist.	43.0	"
		2d "	0.0	"
			<hr/> 43.0	"

For bichromate the danger lies in use of too large a quantity, which gives very low results: probably because of a further oxidation of the acetacetic acid, first formed. This danger may be averted by adding the potassium bichromate in a dilute solution from a dropping funnel during the distillation. At the same time the concentration of sulphuric acid is thereby kept practically constant.

The following results show the effect of varying quantities of potassium bichromate; in these experiments both sulphuric acid and bichromate were added before starting the distillation. The original volume in each distilling flask was about 500 cc. of which about 300 to 350 cc. was distilled.

With 10 cc. H_2SO_4

I.	With 0.3 gm. $K_2Cr_2O_7$	=	40.0	mg. acetone.
II.	0.5 " "		40.0	"
III.	0.5 " "		41.0	"
IV.	1.0 " "		36.0	"
V.	1.0 " "		35.3	"
VI.	3.0 " "		36.8	"
VII.	5.0 " "		23.7	"

With 15 cc. H_2SO_4

I.	With 1 gm. K_2CrO_7		36.2	"
II.	5 " "		23.5	"
III.	10. " "		20.5	"
IV.	20 " (20 cc. H_2SO_4)		13.9	"

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The following are the results of similar determinations except that the bichromate (in dilute solution, usually 0.2 to 0.5 per cent) was added from a dropping funnel during the distillation; the solution dropping in about as fast as the distillate collected.

I.	10 cc. H_2SO_4	0.5 gm. $K_2Cr_2O_7$	40.0 mg. acetone.
II.	10 "	0.5 "	40.0 "
III.	7 "	0.5 "	41.0 "
IV.	7 "	0.5 "	40.0 "
V.	10 "	3.0 "	38.0 "
VI.	10 "	3.0 "	38.4 "
VII.	10 "	3.0 "	36.7 "
VIII.	10 "	3.0 "	38.7 "
IX.	10 "	3.0 "	36.8 "
X.	20 "	3.0 "	38.1 "

In a different solution:

								Mg. acetone per 100 cc. oxy- butyric acid solution.
I.	20 cc. oxybutyric acid sol.	15 cc. H_2SO_4	1 % $K_2Cr_2O_7$					84.0
II.	40 "	"	"	15 "	"	1 "	"	84.5
III.	20 "	"	"	15 "	"	1 "	"	81.5
IV.	40 "	"	"	15 "	"	1 "	"	79.5
V.	20 "	"	"	20 "	"	2 "	"	78.5
VI.	40 "	"	"	20 "	"	2 "	"	79.3
VII.	20 "	"	"	20 "	"	2 "	"	82.5
VIII.	40 "	"	"	20 "	"	2 "	"	83.0
IX.	20 "	"	"	20 "	"	0.1 "	"	82.5
X.	20 "	"	"	20 "	"	2 "	"	79.5

These results show that even when a dropping funnel is used for the addition of the bichromate, the solution should not be too concentrated, or, what is the same thing, the bichromate should be added *slowly*. When this is done the results are accurate. In order to prove this point sodium salt of the inactive acid (already about 90 per cent pure) was prepared and recrystallized three times from absolute alcohol. This recrystallized salt was dissolved in water, excess of sulphuric acid added, cooling with ice, and the solution dehydrated with plaster of paris. The plaster was powdered and extracted with dry ether in a Soxhlet apparatus. The aqueous solution of the ether residue was boiled with pure bone black, filtered, cooled and a portion titrated with $\frac{N}{10}$ alkali (phenolphthalein).

25 cc. = 6.85 cc. $\frac{N}{10} \times 10.4 = 71.2$ mg. β -oxybutyric acid = 39.6 mg. acetone.

Five determinations under identical conditions in 25 cc. portions of this solution gave

40.2 mg. acetone	=	101.3 per cent.
39.1 " "		98.8 "
41.0 " "		103.3 "
40.7 " "		102.7 "
39.2 " "		99.0 "

The distillations were carried out with 15 cc. of concentrated sulphuric acid and keeping the volume nearly constant at about 400 cc. by dropping in water or 0.2 per cent potassium bichromate from a dropping funnel. A total of about 1 gram of potassium bichromate was added, and about 800 cc. distilled in each case.

When this method was applied to urine, difficulties were at once encountered, but were satisfactorily overcome.

Normal urines distilled with chromic acid give acetone although in small quantities. This was shown in 1885 by Flückiger,¹ who found the chief source of the acetone in the conjugated glucuronic acids. The glucuronic acids may however be removed by basic lead acetate and ammonia, which reagents do not precipitate β -oxybutyric acid. The small quantities of formic and butyric acids present in normal and pathological urines would interfere with the determination of β -oxybutyric acid by this method, but these volatile acids may be removed by distilling the urine with sulphuric acid before the addition of bichromate.²

¹ Flückiger: *Zeitschr. f. physiol. Chem.*, ix, p. 343.

² β -Oxybutyric acid in the concentration present during the distillation (never over 0.05 per cent) is not decomposed by 1 per cent to 10 per cent sulphuric acid. Ten cc. β -oxybutyric acid solution + 500 cc. water + 15 cc. sulphuric acid was distilled until 200 cc. distillate had collected. This distillate contained 0.35 cc. $\frac{N}{10}$ acidity, the equivalent of 3.6 mg. of β -oxybutyric acid. The liquid in the distilling flask was again distilled, this time dropping in 0.5 per cent potassium bichromate. This distillate contained 108.4 mg. acetone. Duplicate determinations in the same oxybutyric acid solution, but without previous boiling with sulphuric acid, gave 107.8 mg. and 108.6 mg. acetone. Araki: *Zeitschr. f. physiol. Chem.*, xviii, p 1, 1893) found on distilling 200 cc. of a 1 per cent solution of β -oxybutyric acid that about 1 per cent (0.019 gm.) passed over as crotonic acid in the first 100 cc. distillate.

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Any formic acid passing into the distillate containing the acetone from β -oxybutyric acid would use up iodine in the subsequent titration; while butyric acid, on treatment with bichromate is partially oxidized, presumably to β -oxybutyric acid, thus increasing the yield of acetone.

Glucose also presents obstacles; since on treatment with chromic acid small quantities of what I believe to be formic aldehyde and formic acid are formed. This trouble is very easily overcome by redistilling the distillate supposed to contain acetone from β -oxybutyric acid, with hydrogen peroxide and alkali. The acetone is not attacked¹ but the aldehyde (?) is oxidized to the corresponding acid, which is held back by the alkali.

Lactic acid, when present, also interferes, presumably by the formation of acetic aldehyde which passes over with the acetone, and yields iodoform on treatment with hypoiodite; but this difficulty is likewise wholly removed by subsequent distillation with hydrogen peroxide and alkali.

The following experiments illustrate the above statement concerning glucose and lactic acid.

2 grams glucose in 500 cc. water + 10 gm. sulphuric acid distilled, dropping in 3 per cent potassium bichromate (12 gm.). 600 cc. of the distillate titrated with iodine and thiosulphate gave the equivalent of 19.7 mg. acetone; *but no iodoform was found, the solution remaining clear.*

1 gram glucose in 500 cc. water + 5 cc. β -oxybutyric acid solution + 10 cc. sulphuric acid. Distilled, dropping in 3 per cent potassium bichromate (9 gm.). Distillate = 46.2 mg. acetone. Duplicate = 47.3 mg. acetone.

No glucose, 5 cc. β -oxybutyric acid solution, etc. Distillate = 36.7 mg. acetone. Duplicate = 38.7 mg. acetone.

1 gm. glucose + 50 cc. of a different β -oxybutyric solution + 10 cc. sulphuric acid + water. Distilled, dropping in 2 per cent potassium bichromate. First distillate redistilled with 25 cc. 3 per cent hydrogen peroxide + 5 cc. 10 per cent sodium hydroxide. This distillate = 41.6

¹ Dakin: *This Journal*, iv, p. 81, 1908. The following experiment shows the stability of acetone in the presence of hydrogen peroxide. 25 cc. of a dilute acetone solution titrated direct with standard iodine and thio-sulphate = 36.3 mg. acetone. 25 cc. acetone solution + 400 cc. water + 25 cc. 3 per cent hydrogen peroxide + 5 cc. 10 per cent sodium hydroxide. Distillate contained 36.3 mg. acetone. Duplicate distillate contained 36.9 mg. acetone.

mg. acetone. Control determination in pure solution without glucose = 42.8 mg. acetone.

2.5 gms. calcium lactate in 500 cc. water + 10 cc. sulphuric acid. Distilled, dropping in 2 per cent potassium bichromate. 400 cc. of the distillate gave the equivalent of 80 mg. acetone, with the formation of much iodoform.

1 gm. calcium lactate in 500 cc. water + 25 cc. acetone solution (= 36.3 mg. acetone) + 10 cc. sulphuric acid. Distilled 500 cc., dropping in 2 per cent potassium bichromate (4 gms.)

(a) 125 cc. distillate titrated direct = $18.3 \times 4 = 73.2$ mg. acetone.

(b) 250 cc. distillate redistilled with hydrogen peroxide + sodium hydroxide = $17.9 \times 2 = 35.8$.

(c) Gave strong positive reaction with ammoniacal silver nitrate solution containing sodium hydroxide.

By the use of basic lead acetate and ammonia, and of the second distillation with hydrogen peroxide and alkali it is possible to overcome all of the more serious drawbacks to this method which I have so far encountered.

Phenol and skatol from the conjugated acids may still cause some inaccuracy but the greater part of these substances pass over in the preliminary distillation with sulphuric acid alone; and in any event this error is very small—from 20 to perhaps 100 mg. in a 24-hour urine.

Other substances, such as leucin,¹ sometimes present in urine in relatively small quantities, may be capable of forming acetone under the circumstances, but it is not likely that this possible error is of sufficient size to materially detract from the usefulness of the method.

A number of different normal and pathological urines, not containing β -oxybutyric acid, which I have examined have given results equivalent to less than 0.100 gram β -oxybutyric acid for a 24-hour urine (0.010 gram to 0.080 gram).

In the routine use of this method we determine at the same time the preformed acetone plus acetone from diacetic acid, with very satisfactory results. The three distillations of the Meslinger-Huppert method are for practical purposes unnecessary, though two distillations are desirable.

The method which I propose for the determination of acetone + diacetic acid, and of β -oxybutyric acid is carried out as follows:

¹ Dakin: This *Journal*, iv, p. 63, 1908.

220 Determination of β -Oxybutyric Acid

From 25 to 250 cc. of urine, depending upon whether much or little β -oxybutyric acid is expected,¹ is measured into a 500 cc. volumetric flask and an excess of basic lead acetate and 10 cc. of concentrated ammonium hydroxide are added. This is diluted to the mark with water, shaken and filtered. An aliquot part of the filtrate (usually 200 cc.) is diluted with water to 500 to 600 cc., 15 cc. of concentrated sulphuric acid and talcum added, and the mixture distilled until 200 to 250 cc. of distillate has collected. (Distillate A.)

The distilling flask (800 cc. Kjeldahl's are convenient) must be fitted with a dropping tube and water run in to prevent the volume in the flask from becoming less than 400 cc.

Distillate A contains the acetone, preformed and from diacetic acid, and also the volatile fatty acids present; to remove the latter, including the disturbing formic acid, distillate A is redistilled after adding a little fixed alkali (5 cc. of 10 per cent sodium hydroxide). This distillate (A_2) is titrated with standard iodine and thiosulphate.

The residue of urine + sulphuric acid from which Distillate A was obtained is again distilled, dropping in 400 to 600 cc. of 0.1 per cent to 0.5 per cent potassium bichromate solution. (Ordinarily 0.5 gm. potassium bichromate will be sufficient; with much sugar or when much urine is used even 2 or 3 gms. may be necessary.) The potassium bichromate solution should not be added faster than the distillate collects unless the boiling liquid turns pure green, thus indicating that the bichromate is being used up more rapidly. When about 500 cc. of distillate (B) has collected, 20 cc. of 3 per cent hydrogen peroxide is added to the distillate (or the distillate may be collected in a flask containing the hydrogen peroxide) together with a few cc. of sodium hydroxide solution and this distillate (B) is again distilled. This dis-

¹ Of diabetic or other urines giving a strong ferric chloride reaction for diacetic acid, or when 5 to 10 grams or more β -oxybutyric acid is expected, 25 to 50 cc. or even less urine will be found a suitable quantity; when little or no β -oxybutyric acid is expected, 125 cc. or 250 cc. may be used. In either case the amount taken is sufficient for duplicate determinations. The aim should be to use such a quantity of urine as will give between 25 and 50 mg. of acetone from β -oxybutyric acid.

tillate now obtained (B_2 ; 300 cc. should be distilled) is titrated as usual with iodine and thiosulphate.

A good condenser must be used for the distillations, but it is not necessary to cool the distillates with ice, as is sometimes recommended.

We find it convenient to make our thiosulphate and iodine solution 103.4 per cent $\frac{N}{10}$. Each cc. of the iodine solution is then equal to 1 mg. of acetone or to 1.794 mg. of β -oxybutyric acid. The thiosulphate is accepted as the standard, and is restandardized from time to time by $\frac{N}{10}$ potassium bi-iodate.

A few determinations in urines containing no β -oxybutyric acid and also of normal urines to which were added varying amounts of synthetic β -oxybutyric acid, are given below.

Lab. No. 622 Mixed urine of 5 days from a case of epilepsy. Average for 24 hours.

Acetone + diacetic	=	0.018 gm. acetone.
β -Oxybutyric acid	=	0.018 "
		0.025 "

Lab. No. 626. 24-hour urine from a case of Grave's disease. 1220 cc sp. gr. 1.017.

Totals: Acetone + diacetic	=	0.015 gm. acetone.
β -Oxybutyric	=	0.025 "

Lab. No. 637. 24-hour urine from woman 5 months pregnant.

Totals: Acetone + diacetic	=	0.032 gm. acetone.
β -Oxybutyric	=	0.032 "

Lab. No. 638. 24-hour urine from woman 7 months pregnant, who had a moderate acidosis some weeks earlier.

Acetone + diacetic	=	0.006 gm. acetone.
β -Oxybutyric	=	0.007 "

Lab. No. 641. Specimen of urine representing probably about 12 hours from a case of puerperal eclampsia. Urine passed about the time of the convulsions 400 cc., sp. gr. 1.019; 0.25 per cent albumen.

Acetone + diacetic	=	0.027 gm. acetone.
β -Oxybutyric	=	0.042 "

Mixed normal urine.

For 1000 cc.: β -oxybutyric = 0.025 gm. acetone.

To the same urine was added 1.50 gm. β -oxybutyric acid per liter of urine.

222 Determination of β -Oxybutyric Acid

For 1000 cc. of urine, β -oxybutyric acid = 0.860 gm. acetone.

"	"	"	"	"	"	0.865	"
"	"	"	"	"	"	0.890	"
"	"	"	"	"	"	0.860	"

Average = 0.869 gm. acetone.

= 1.56 gm. β -oxybutyric acid.

Determinations in same β -oxybutyric acid solution: (a) 0.825 gm.;
(b) 0.830 gm.; (c) 0.840 gm.; (d) 0.840.

Average = 0.834 gm. acetone,

= 1.50 gm. β -oxybutyric acid.

Normal urine: sp. gr. 1.021.

Results are given in grams of acetone per 1000 cc. of urine.

I. Without treatment with basic lead acetate and ammonia.

Acetone + diacetic: Distillate A titrated direct = 0.060 gm.

Distillate A redistilled with
sodium hydroxide = 0.016 "

β -Oxybutyric: Distillate B titrated direct = 0.28 "

Distillate B redistilled with
hydrogen peroxide and
sodium hydroxide = 0.18 "

II. After treatment with basic lead acetate and ammonia.

Acetone + diacetic: Distillate A titrated direct = 0.042 "

Distillate A redistilled with
sodium hydroxide = 0.008 "

β -Oxybutyric: Distillate B titrated direct = 0.046 "

Distillate B redistilled with
hydrogen peroxide and
sodium hydroxide = 0.018 "

III. Same urine + β -oxybutyric acid solution (2000 cc. β -oxybutyric acid solution contained according to determinations in the solution 0.686 gm. acetone). 2000 cc. of β -oxybutyric solution to 1000 cc. urine. Treated with basic lead acetate and ammonia.

Acetone + diacetic: Distillate A titrated direct = 0.036 "
0.036 "

Distillate A redistilled with
sodium hydroxide. = 0.010 "

β -Oxybutyric: Distillate B titrated direct = 0.706 "
0.668 "

Distillate B redistilled with
hydrogen peroxide and
sodium hydroxide = 0.670 "
0.634 "

- IV. Same proportions of urine and β -oxybutyric acid as in III; 8 gms. glucose added per 100 cc. urine. Treated with basic lead acetate and ammonia.

Acetone + diacetic: Distillate A redistilled with sodium hydroxide = 0.016 gm.

β -Oxybutyric acid: Distillate B redistilled with H_2O_2 + N_2OH = 0.664 "

- V. Same as III, but 4 gms. calcium lactate added per 100 cc. urine. Treated with basic lead acetate and ammonia.

Acetone + diacetic: Distillate A titrated direct = 0.042 "
Distillate A redistilled with sodium hydroxide = 0.022 "

β -Oxybutyric: Distillate B titrated direct = 2.338 "
Distillate B redistilled with hydrogen peroxide and sodium hydroxide = 0.664 "

- VI. Same urine + one-half the amount of β -oxybutyric acid (= 0.343 gm. acetone per 1000 cc. urine, according to previous determinations in pure solution). Treated with basic lead acetate and ammonia.

Acetone + diacetic: Distillate A redistilled with sodium hydroxide. = 0.014 "

β -Oxybutyric: Distillate B redistilled with hydrogen peroxide and sodium hydroxide. = 0.330 "
0.334 "
0.340 "

Lab. No. 627. Diabetic urine, 1710 cc., sp. gr. 1.025. Determinations made as described on p. 220.

Total acetone + diacetic = 0.81 gm. acetone.

Total β -oxybutyric = 2.00 gms. acetone or 3.60 gms. β -oxybutyric acid.

If 250 cc. of this urine (= 0.525 gm. β -oxybutyric acid) were used for a determination by any of the ether extraction methods, the ether residue dissolved in 50 cc. water and the resulting 1.05 per cent solution read in a 200 mm. tube in the polariscope, an error of 0.05° in reading would be 9.5 per cent of the total.

I am indebted to Mr. E. A. Reinoso for carrying out many of these experiments.

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THE EXCRETION OF KREATININ AND KREATIN IN HEALTH AND DISEASE.

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IT is the purpose of this paper to present results on the excretion of kreatinin and of kreatin by normal and by pathological subjects, in the hope of throwing some further light upon the physiological significance of these two substances.

Previous to 1904 the Neubauer method¹ of precipitation with zinc chloride was used for the determination of kreatinin; while for the determination of kreatin the substance was isolated as such and weighed. In neither case are the results reliable from a quantitative standpoint.

A complete review in this paper of the observations made by the use of these methods would serve no purpose, since the literature of this subject has been fully treated by recent writers.² The more important of these observations may, however, be again stated very briefly.

¹ NEUBAUER: *Annalen der Chemie und Pharmacie*, 1861, cxix, p. 33; SALKOWSKI: *Zeitschrift für physiologische Chemie*, 1886, x, p. 113; SALKOWSKI and TANIGUTI: *Ibid.*, 1890, xiv, p. 471.

² VON NOORDEN'S *Handbuch der Pathologie des Stoffwechsels*: HOOGENHUYZE and VERPLOEGH: *Zeitschrift für physiologische Chemie*, 1905, xlv, p. 415; MELLANBY: *Journal of physiology*, 1908, xxxvi, p. 447.

Acid human urine contains kreatinin, and little or no kreatin; an alkaline urine contains kreatin instead of kreatinin (Voit³). The amount of kreatinin excreted by an adult was found to vary between 0.4 gm. and 1.5 gm. (Voit,³ Munk,⁴ Neubauer,⁵ Hofmann⁶), and was believed to depend upon the amount of protein as well as upon the amount of kreatin (Gruber,⁷ Hofmann,⁶ Meissner,⁸ Mallet⁹) in the food. Regarding other factors, Hofmann⁶ found that the amount of kreatinin excreted increases with growth, and declines in old age; that the amount excreted by women is somewhat lower than that of men; that body height has no influence, and that there is some relationship between body weight and the amount of kreatinin excreted, but apparently he did not consider the last-mentioned point of great importance, since he did not give the weights of any of his subjects.

In view of the close chemical relationship between kreatin and kreatinin and of the supposed ease with which one is converted into the other, it is very generally believed, but without any definite proof, that the kreatinin of the urine is derived from the kreatin of the muscles.

Conflicting statements are to be found concerning the influence of muscular work. Moitessier,¹⁰ Grocco,¹¹ and Gregor¹² found an increased excretion of kreatinin following muscular work, while Hofmann⁶ and Meissner⁸ reached the opposite conclusion.¹³

With pathological individuals and using the Neubauer method, Hofmann,⁶ Schottin,¹⁴ and Munk,⁴ found an increased excretion in

³ VOIT: *Zeitschrift für Biologie*, 1868, iv, p. 77.

⁴ MUNK: *Deutsche Klinik*, 1862, No. 30, p. 299.

⁵ NEUBAUER: *Annalen der Chemie und Pharmacie*, 1862, cxx, p. 27.

⁶ HOFMANN: *VIRCHOW'S Archiv für pathologische Anatomie*, 1869, xlviii, p. 358.

⁷ GRUBER: *Zeitschrift für Biologie*, 1901, xlii, p. 416.

⁸ MEISSNER: *Zeitschrift für rationelle Medicin*, 1865-68, xxiv, xxvi, and xxxi.

⁹ MALLET: *Bulletin No. 66*, 1899, U. S. Office of Experiment Stations.

¹⁰ MOITESSIER: *Thesis*, 1891. Cit. by Hoogenhuyze and Verploegh.

¹¹ GROCCO: *Cit. MALY'S Jahresbericht*, 1886, xvi, p. 199.

¹² GREGOR: *Zeitschrift für physiologische Chemie*, 1900, xxxi, p. 98.

¹³ I need not refer here to the recent experiments on this point. By the use of the FOLIN method it has been shown that neither an increased (HOOGENHUYZE and VERPLOEGH) nor a decreased (SHAFFER) muscular activity has any effect upon the amount of kreatinin excreted.

¹⁴ SCHOTTIN: *Cit. by KRAUS in VON NOORDEN'S Handbuch der Pathologie des Stoffwechsels*, 1906, i, p. 137.

fevers. Low results were found in chronic under-nutrition (Hofmann⁶); in convalescence (Munk⁴); in chlorosis (Hofmann⁶); in pernicious anæmia, in myelogenous leucæmia, and in lymphatic leucæmia (Stejskall and Erben¹⁵); in pseudo leucæmia (Moraczewski¹⁶); and in muscular atrophy (Weiss, Jakubowitsch, Langer¹⁷). A high excretion of kreatinin was noted in diabetes (Senator, Gathgens, and others¹⁸), probably because of the kreatin and kreatinin in the meat eaten.

But on account of the inaccuracy of the analytical method used and of the great variations found in the amounts of kreatinin excreted by normal persons, these pathological results have lacked any great significance, and must now be accepted with caution.

With the advent of Folin's quick and relatively accurate method¹⁹ for the determination of kreatinin and kreatin, interest concerning these substances was greatly revived; in consequence, our knowledge concerning them has been materially advanced, and in several important instances the conclusions of earlier investigators as stated in the foregoing pages have already been disproved.

Folin first showed that the amount of kreatinin excreted in the urine by a normal individual is, contrary to Hofmann's conclusion, quite independent of the amount of protein in the food, or of total nitrogen in the urine; the amount of kreatinin excreted from day to day is practically constant for each individual (presumably under the same conditions of health and muscular activity).²⁰

This constancy of kreatinin excretion has been fully confirmed by Hoogenhuyze and Verploegh,²¹ Klercker,²² Closson,²³ and the

¹⁵ STEJSKALL and ERBEN: *Zeitschrift für klinische Medicin*, 1900, xxxix and xl.

¹⁶ MORACZEWSKI: *VIRCHOW's Archiv für pathologische Anatomie*, 1898, cli, p. 22.

¹⁷ WEISS: *Wiener klinische Wochenschrift*, 1877, p. 701; JAKUBOWITSCH: *Neurologisches Centralblatt*, 1884, p. 279; LANGER: *Deutsche Archiv für klinische Medicin*, 1883, xxxii, p. 395.

¹⁸ Cit. by VON NOORDEN: *Handbuch der Pathologie des Stoffwechsels*, 1907, ii, p. 90.

¹⁹ FOLIN: *Zeitschrift für physiologische Chemie*, 1904, xli, p. 223.

²⁰ FOLIN admitted that the amount excreted might be dependent upon the amount of muscular work (*This journal*, 1905, xiii, p. 86).

²¹ HOOGENHUYZE and VERPLOEGH: *Zeitschrift für physiologische Chemie*, 1905, xlvi, p. 415.

²² KLERCKER: *Biochemische Zeitschrift*, 1907, iii, p. 45.

²³ CLOSSON: *This journal*, 1906, xvi, p. 252.

writer.²⁴ If the subject is receiving sufficient food, the kreatinin excretion is the same with 16 gm. as it is with 4 gm. total nitrogen in the urine. Folin²⁵ and Klercker²² further showed that, contrary to the previously accepted views, the excretion of kreatinin is wholly unaffected by the ingestion of kreatin; and this result has been confirmed by Wolf and the writer.²⁶

Since kreatinin, unlike any other known product of normal metabolism excepting perhaps uric acid, is independent of the total amount of protein katabolized, it appears to be of the greatest interest to learn the full significance of kreatinin, and to determine further the factors which may influence the amount of this substance excreted in the urine.

THE NORMAL EXCRETION OF KREATININ.

While the kreatinin excretion is practically constant from day to day for each healthy individual, different persons excrete different amounts; and Folin pointed out that "the chief factor determining the amount of kreatinin eliminated appears to be the weight of the person."²⁷ He further realized that the amount of adipose tissue must be considered, because he noted that the fatter the subject the less kreatinin is excreted for each kilo of body weight. Folin considered these points rather briefly, but the conclusion from his results is that the amount of kreatinin excreted depends primarily upon the mass of active protoplasmic tissues.

Since we have no method of accurately measuring the amount of adipose tissue in various individuals, the best that we can do is to express the amount of kreatinin excreted as milligrams kreatinin, or, as seems to me preferable, as milligrams kreatinin-nitrogen per kilo of body weight. This ratio, milligrams kreatinin-nitrogen per kilo of body weight, I have called the "kreatinin coefficient,"²⁸ and this term will be used in the following pages.

²⁴ SHAFFER: This journal, 1908, xxii, p. 445.

²⁵ FOLIN: HAMMARSTEN'S Festschrift, 1906, iii, p. 1.

²⁶ WOLF and SHAFFER: Journal of biological chemistry, 1908, iv, p. 439.

²⁷ FOLIN: This journal, 1905, xiii, p. 85.

²⁸ "The effect of muscular activity on kreatinin excretion; with preliminary observations on the excretion of kreatinin in health and disease," Proceedings of the American Physiological Society, New York, Dec. 1906. In this paper the kreatinin coefficients were expressed as milligrams kreatinin, but I have since

Table I contains averages of kreatinin values determined by Folin's method, in the urine of supposedly normal persons; most of these results have been taken from the literature, only the last ten being my own. Almost all of the figures are averages from several days and in some cases represent much longer periods. These figures contain evidence in favor of the factors, body weight and amount of adipose tissue, suggested by Folin, as influencing the kreatinin excretion. But these are certainly not the only factors of influence, and because of the complexity of the subject it is difficult to show the effect of any one factor alone.

These figures, covering 37 supposedly normal cases, show an average of 8.1 with a maximum of 11.7 and a minimum of 5.4. These we may for the present accept as the normal limits for kreatinin excretion,²⁹ although I believe that a closer analysis and further experience will show that kreatinin coefficients below 7 are normal only for elderly, inactive, poorly developed, or excessively fat subjects, and, strictly speaking, none of these conditions is normal.

HOURLY EXCRETION OF KREATININ.

In view of the remarkable uniformity in the excretion of kreatinin from day to day, it seemed desirable to learn the extent of variation during various periods in the twenty-four hours. A large number of determinations in the urine of seven subjects showed just as great uniformity in the hourly excretion of kreatinin as is found in the daily excretion. A portion of these results is given in Table II.

The time of each period was exactly noted, and an effort was made to empty the bladder as completely as possible each time in order to get quite all of the urine secreted in the time stated. No meat products were contained in the diets except where noted in the table.

concluded that it will be more consistent, and in the end less confusing, to express the kreatinin coefficient, like results of other nitrogenous substances, in terms of nitrogen.

²⁹ The kreatinin coefficients of normal dogs are practically the same as those given above for man. WOLF and OSTERBERG (*Biochemische Zeitschrift*, 1907 v, 304) found an average of 8.2 for one dog (fourteen days) and 7.0 for another (thirteen days). Results of my own from four dogs lie between the above figures. The amount of kreatinin excreted from day to day is as constant for dogs as for men (OSTERBERG and WOLF, *loc. cit.*, and unpublished experiments of the writer).

It is by no means an easy matter without some practice to empty the bladder completely, especially at frequent intervals, and it is not unlikely that at least some of the slight variations found are to be explained by this difficulty. Where this is so, a low result will of course precede or follow a high result.

The lack of effect of diuresis on the excretion of kreatinin is shown in some of these figures.³⁰ With subject M. S. the volume of urine varied from 24 c.c. to 934 c.c. per hour, the latter after taking 2 gm. diuretin, but the amount of kreatinin excreted in each period was practically the same (0.059 and 0.054 gm.). The same thing is shown in the results from P. A. S. Between 1 and 2 P.M. on one day, for instance, 432 c.c. of urine was secreted, containing 68 mg. of kreatinin; the next hour only 66 c.c. of urine was secreted, but this contained 70 mg. kreatinin.

A great increase or decrease in the amount of protein ingested at a single meal, resulting in marked change in the amount of total nitrogen excreted per hour, is also without effect upon the hourly excretion of kreatinin. (See the results from M. S.)

These results appear to show that the regularity of excretion of kreatinin is to be explained by a regularity of formation, and not merely by a regular secretion by the kidneys.

There are slight variations during the twenty-four hours, but the results are, on the whole, remarkably regular, and justify the belief that kreatinin is formed during a process in the body which varies very little in intensity from hour to hour.

The papers by Hoogenhuyze and Verploegh and by Klercker contain data on the hourly excretion of kreatinin; but their results do not show any such regularity. The greater variations found by these investigators are possibly due to some inaccuracy in the length of periods, etc.; in any event, my results show that there is a far greater regularity in the hourly excretion than their observations indicate.

NORMAL EXCRETION OF KREATIN.

As mentioned earlier in this paper, Voit believed that an alkaline urine contains kreatin instead of the kreatinin which he found when

³⁰ When less than about 8 mg. of kreatinin was contained in 25 c.c. urine, the latter was made slightly more acid with HCl, and evaporated to a smaller volume before the determination. Except on rare occasions and for very small quantities, kreatin was not present in these urines.

the urine was acid. This is not correct. Normal fresh urine, whether acid or alkaline, contains kreatinin, and if the normal subject has not taken kreatin in his food during the preceding few days, his urine will not contain kreatin, whatever its reaction. There is no normal excretion of endogenous kreatin.³¹

EXCRETION OF KREATININ BY PATHOLOGICAL SUBJECTS.

During the past three years we have been carrying on in this laboratory metabolism experiments upon various hospital patients, many of whom were in bed. From these subjects we have found, very frequently, much lowered kreatinin coefficients, notwithstanding the fact that many of the patients were more or less emaciated, and therefore, because of the little adipose tissue, we might perhaps have expected high kreatinin coefficients. A number of times it has been further observed that when patients improved, got out of bed, and walked around, the kreatinin coefficient was raised to some extent. When I referred to some older results³² from patients at the McLean Hospital for the Insane, many of whom were not in bed and had no definite disturbance of their vegetative organs, but who were not taking much physical exercise, I found again many instances of a much lowered kreatinin coefficient.

These findings seemed at first to point clearly in one direction,—that the lowered kreatinin excretion was the result of the small amount of muscular energy expended by such individuals. I therefore proceeded to determine the effect of muscular activity, particularly of a greatly diminished activity, on kreatinin excretion, and, incidentally, upon the general protein metabolism.

These experiments, which are described in a separate paper,³³ led to the conclusion that the amount of muscular activity is in itself wholly without effect upon the amount of kreatinin excreted. We may, therefore, leave out of consideration the factor of muscular work. Some other explanation must be found for the very low kreatinin excretion noted in abnormal subjects.

We have made, in this laboratory, observations on the kreatinin

³¹ FOLIN has shown that kreatin, when ingested, is largely retained in the body unless the food contains a large amount of protein (HAMMARSTEN'S *Festschrift* iii). See also KLERCKER, *loc. cit.*

³² FOLIN: *American journal of insanity*, 1904, xli, p. 299.

³³ SHAFFER: *This journal*, 1908, xxii, p. 445.

excretion of more than two hundred different persons, representing a variety of conditions. Folin's method has been used exclusively. The lowest kreatinin coefficient (2.4) was found in a subject of lymphatic leucæmia, who was extremely ill and died about a week later. Coefficients of 3 or 4 are comparatively common in patients who are confined in bed on account of weakness, whatever may be the disease. From these low values the coefficients vary up to the normal figures, 8-11. In subjects of acute fevers, in the early stages, the excretion of kreatinin is quite normal or high, but with the disappearance of the fever the kreatinin falls to below the normal.³⁴ The publication of all our results is unnecessary; a few will be found at the close of this paper, while some others will be given in forthcoming papers from this laboratory.

The facts which I wish to emphasize in this paper are that *a low excretion of kreatinin is found in a remarkably large number of pathological subjects, representing a variety of conditions, and that the excretion of an abnormally small amount of this substance is by no means peculiar to any one disease.*

What is the significance of low kreatinin excretion? According to Folin,³⁵ the kreatinin excreted, on a kreatinin-free food, is an index of and wholly derived from endogenous or tissue katabolism. Folin has not defined his "tissue katabolism," but if we understand that term to cover all of the processes taking place in the cells of the body in which the body protein is broken down, the *total* endogenous katabolism, we shall be compelled to modify this idea of the origin of kreatinin.

At a meeting of the American Physiological Society (New York, December, 1906), after comparing normal results, as given in a previous chapter of this paper, with low results from a number of pathological cases, I suggested that the kreatinin of the urine is derived from, and an index of, not the total tissue or endogenous katabolism, but of one process of this tissue katabolism; and I then pointed out that on this latter process appears to depend the muscular, or general cellular, efficiency.

³⁴ LAMBERT and WOLF: Proceedings of the American Society of Biological Chemists, 1907, i, p. 28; FOLIN: unpublished results, personal communication; SHAFFER: unpublished results; LEATHES: Journal of physiology, 1907, xxxv, p. 205.

³⁵ FOLIN: This journal, 1905, xiii, p. 84.

In May, 1907,³⁶ I presented further low kreatinin coefficients from subjects of exophthalmic goitre, whose total endogenous metabolism was above the normal, as shown by rapid loss of weight and emaciation; and the conclusion was again drawn, "that kreatinin is not a product of total tissue catabolism, but is a product of certain *normal* cell processes, which in many diseased conditions may be extremely sluggish in their intensity, even though, as in exophthalmic goitre, the total tissue catabolism may be much increased. The low kreatinin coefficients in all marked cases of exophthalmic goitre — subjects of which disease are especially prone to muscular weakness — are also accepted in support of the author's hypothesis that kreatinin is an index of muscular tonus, or of muscular and perhaps of general cellular efficiency."

Whether kreatinin arises in this katabolic process in all of the tissues of the body, or whether it is alone formed in the muscles, cannot be decided without further experiments;³⁷ but for the muscular tissues, at any rate, facts support the belief that the amount of kreatinin excreted is an index of their efficiency, — not the amount of work which the muscles are doing at the time, but the amount of work which they are capable of doing.

In October, 1907, a paper appeared by Spriggs,³⁸ who, using Folin's method, came to essentially the same conclusion. Spriggs found the kreatinin excretion very low (2.2 and 4 mg. kreatinin nitrogen per kilo body weight) in two cases of muscular dystrophy (decrease of muscular bulk); very low (1.9 mg. kreatinin-nitrogen per kg.) in a case of amyotonia congenita; slightly low (5.6 mg.) in a case of myasthenia gravis; normal in one case of locomotor ataxy (7.1); and slightly high (?) in two cases of tetanus (7.8 and 9.3).

Spriggs concluded from these cases that "kreatinin is connected with the nutritional metabolism of the muscle fibre, and is not a

³⁶ SHAFFER: Proceedings of the American Society of Biological Chemists, 1907, i, p. 22.

³⁷ The experiments of GOTTLIEB and STANGASSINGER (*Zeitschrift für physiologische Chemie*, lii and lv) appear to indicate that the formation of kreatin and kreatinin takes place in the glandular organs as well as in the muscles. This phase of the subject, the site of formation of kreatinin, and its possible relation in the body to kreatin will be treated of in a future paper. Experiments on these points by Dr. R. A. HATCHER and the writer were begun over a year ago and are still in progress.

³⁸ SPRIGGS: The quarterly journal of medicine (Oxford), 1907, i, p. 63.

substance formed in the act of contraction. If we liken the muscles to a machine, creatinin as a waste product would stand in relation to the structure of the machine, and not to the fuel which the machine uses." This statement is quite in accord with my own views, as presented to the American Physiological Society and to the American Society of Biological Chemists.

If this idea is correct, it merely means that in a muscle in a high state of nutrition and development certain processes which cannot be at present fully defined, but which lead to the formation of kreatinin as a waste product, are proceeding at a greater speed than in a muscle organically weak or diseased.

The actual efficiency of a muscle depends upon a number of factors, one of which is the nerve impulse sent to that muscle; but my view in this connection, and as I understand it the view held by Spriggs, considers the muscle only as an individual machine, and as being quite independent of nervous stimulation. If the motor nerve to one of the skeletal muscles is cut, that muscle is actually unable to work, but on account of the lack of a stimulus, and not because of any inefficiency of the muscle fibres. Potentially the muscle is as good as before. This applies of course only to the immediate effects; the nervous mechanism being destroyed or diseased, the muscle becomes atrophied from disuse, its nutrition is interfered with, and then, as a secondary effect, the efficiency of the muscle as a machine is decreased. I believe it is only this secondary effect which under these conditions would lead to a decreased excretion of kreatinin.

I should not expect temporary anæsthesia, for instance, to cause any marked decrease in kreatinin excretion, although it would for the time destroy the actual muscular efficiency; a long illness or old age, on the other hand, which leaves the person muscularly weak should, and does, cause a corresponding decrease in the amount of kreatinin excreted.

Some reservation must be made for the effect of fever, which probably increases, though in my experience not greatly, the excretion of kreatinin; and it cannot be supposed that fever increases temporarily the efficiency of the muscles. The greater excretion in fever may be ascribed to a pathological increase of the kreatinin forming process, which is perhaps due solely to the higher temperature or to the action of bacterial toxins, and which is coincident with the increased destruction of body protein. For non-febrile

individuals my results indicate that the amount of kreatinin excreted bears a direct relation to the potential efficiency of the muscles, and is a reliable index of the muscular development of an individual.

A number of the variations in the kreatinin coefficients of normal persons (Table I) may be explained by a consideration of the varying muscular efficiency of the subjects. Of those of the individuals known to me, the ones with the better muscular development and capable of the greater amount of muscular work have the higher kreatinin coefficients, and *vice versa*.

The idea outlined above has already received some consideration at the hands of other workers. Benedict and Meyers³⁹ report determinations of kreatinin in the urine of twenty-six women, all of whom were patients in a hospital for the insane. Most of these cases, it seems to me, fully bear out the factors above outlined as influencing the amount of kreatinin excreted and its relation to muscular efficiency. I shall cite only the high and low extremes in these cases.

Case I was a female, 85 years old, senile dementia, and weighed 39 kg. "Subject in bed, old, feeble, withered, and very inactive." Kreatinin coefficient, 2.0.⁴⁰

Case XVI. Female, age 92 years, weight 63 kg. "Subject rather decrepit, partly paralyzed, fat and flabby. Spends most of time in bed." Kreatinin coefficient, 2.0.

This case was doubtless muscularly stronger than Case I, since she was out of bed a part of the time. With relatively the same amount of adipose, she should have had a higher coefficient than Case I; but she was also "fat and flabby," and in consequence had relatively less muscular tissue than Case I.

Case XXIII. A nurse, 25 years old, weighing 52 kg., just convalescing from typhoid fever, and was considerably under normal body weight." Kreatinin coefficient, 3.1.

In convalescence from typhoid there is usually a marked muscular weakness.

Some of these cases had coefficients near the normal.

³⁹ BENEDICT and MEYERS: This journal, 1907, xviii, p. 377.

⁴⁰ BENEDICT and MEYERS express the kreatinin coefficients in mg. kreatinin; for comparison with my results I have converted their figures into mg. kreatinin-nitrogen.

Case XIII. Manic depressive insanity, depressed form, "in a fair physical condition and extremely active." Kreatinin coefficient, 5.6.

Case XXII. Dementia præcox, age 45 years. "Subject rather inert as a rule," in bed because of erysipelas. Kreatinin coefficient, 5.9.

Benedict and Meyers conclude that "the kreatinin excretion of women is, in general, much lower than that of men." This is doubtless true, and is to be explained by the fact that most women are poorer developed muscularly than men; the kreatinin-forming process is less active, and they have at the same time a lower muscular efficiency.

I have had the opportunity of determining the kreatinin coefficients of only a few strong and hearty women, but these and my results from pathological subjects indicate that if the factors of muscular development or efficiency and the amount of adipose are nearly the same, there is no difference between the kreatinin coefficients of men and women. Sex, *per se*, has, I believe, no influence.

The results of Amberg and Morrill⁴¹ are in agreement with my hypothesis. These investigators find from very young infants kreatinin coefficients between 1.46 and 2.6 (mg. kreatinin-nitrogen). These figures are what should be expected from the smaller bulk of muscle tissue, and the low muscular tonus of infants only a few days old.

In experiments upon a fasting dog, poisoned with phosphorus, Lusk found "a gradual fall in the amount [of kreatinin] eliminated — independent of the tone and strength of the muscle," but states in his summary that "the kreatinin output is scarcely affected."⁴²

A calculation from his results shows that the kreatinin coefficient of the dog on the third day of the fast was 7.0, which fell to 6.3 on the sixth day, and to 5.5 on the seventh day. A dog's muscles are certainly less efficient on the seventh day of a fast than on the third day. The subsequent rise of the kreatinin excretion after the injection of the phosphorus is doubtless due to the toxic action of the latter, and, I think, speaks neither for nor against my hypothesis. Such a toxic increase is quite analogous to that seen during fever. On the other hand, it should not be concluded, because the dog was severely ill from the poisoning with phosphorus, and I was unable to stand that his muscles, considered as individual machines, lost

⁴¹ Ayres and Moore: *Journal of Biological Chemistry*, 1907, 11, p. 311.

⁴² *Ibid.* *Trans. normal*, 1907, 11, p. 312.

in potential efficiency to a corresponding degree. It seems to me more probable that the muscular weakness of this dog was due more to a depression of the nervous mechanism, and not to any great extent to a decreased efficiency of the muscles themselves.

Mention must also be made of the recent admirable paper by Mellanby⁴³ on "Creatin and Creatinin." This author presents a large number of valuable facts concerning the kreatin content of muscles from many different animals, and concludes that "in the formation of kreatinin muscle plays a small part," and that "the liver is intimately connected with the production of kreatin and the excretion of kreatinin." He introduces distinctly a new point of view in believing that the liver forms kreatinin from other substances, that this kreatinin is in part converted into kreatin and stored in the muscles until the amount of kreatin in the muscles reaches the "saturation point," after which the excess of kreatinin, continually being formed by the liver, is excreted in the urine.

According to Mellanby's idea one would expect a low kreatinin excretion from subjects of diseases interfering with the normal function of the liver; and he presents such data. But subjects of cirrhosis and other pathological conditions of the liver are far from being the only instances of low kreatin excretion, and in view of the large number of subjects having low kreatinin-coefficients, and in whom there is no reason for suspecting any disturbance or diminution of liver function, it does not appear to me probable that there can be such a relation between the activity of the liver and the formation and excretion of kreatinin as he suggests. The relationship between body weight and the amount of kreatinin excreted may also be mentioned as an argument against the correctness of his idea.

KREATIN.

As stated earlier, kreatin is not present in normal urines unless kreatin is taken in the food. This substance is, however, excreted in various pathological conditions even when the food is free from kreatin. It is logical to suppose, as do Benedict⁴⁴ and Mellanby, that the kreatin excreted has its source directly in the kreatin of the muscles; and this I believe to be true in view of the fact that, according to my observations, kreatin is invariably excreted where

⁴³ E. MELLANBY: *Journal of physiology*, 1908, xxxvi, p. 447.

⁴⁴ BENEDICT: *This journal*, 1907, xviii, p. 406.

there is a rapid loss of muscle protein. It is excreted in acute fevers, in the acute stages of exophthalmic goitre, and in tumor cachexia, in all of which conditions emaciation is taking place, with probably a breaking down of muscle tissue.

The largest excretion of kreatin which I have so far encountered is in women during the first week *post partum*, when I have found as much as 1.50 gm. (as kreatin) in twenty-four hours; it is during this stage that the resolution of the muscular wall of the uterus is proceeding most rapidly. It may also be excreted even when the body is increasing in weight, as I have observed in a case of exophthalmic goitre who was improving rapidly. But it is conceivable that this may be explained by the persistence in the muscles of a pathological katabolism, otherwise masked by the regenerative processes going on at the same time.

The following are given as instances of kreatinin excretion and of kreatin excretion in pathological individuals. The figures are in nearly all cases averages of a considerable number of days. The diets were always free from kreatin and kreatinin.

Kreatinin-nitrogen is abbreviated to $K_1\text{-N}$, kreatin-nitrogen to $K_2\text{-N}$, and kreatinin-coefficient to $K_1\text{-Coef}$.

Subject	Age	Weight	$K_1\text{-N}$	$K_1\text{-Coef}$	$K_2\text{-N}$
I. A. N. Exophthalmic goitre. Female. Large frame, but had lost about 7 kg. some months earlier. Able to move about the house, but with effort. A little later was much weaker than during the experiment.	35	59 kg.	0.22 gm.	3.7	0.03
II. A. M. H. Exophthalmic goitre. Female. Slow onset, marked symptoms. In bed, not emaciated.	38	49 "	0.163 "	3.3	0.12
Improved, able to be out of doors.	0.217 "	4.4	0.03
After greater improvement. Much stronger.	0.273 "	5.5	0.17
III. Perpall. Exophthalmic goitre. Female. Extremely ill, emaciated to last degree. Died several weeks later.	..	35	0.11 "	3.2	0.11
IV. Redlin. Exophthalmic goitre. Female. Extremely ill. Lost much weight during last year.	18	41 "	0.13 "	3.2	0.12
V. Mrs. J. Exophthalmic goitre. Female. Rapid onset, acute case. Very weak and losing weight.	39	50 "	0.14 "	2.8	0.18
VI. Duffy. Exophthalmic goitre. Female. Severe case. Extremely emaciated and not able to sit up.	..	45 "	0.166 "	2.6	0.11

Subject	Age	Weight	K ₁ -N	K ₁ -Coef.	K ₂ -N
Three months later after very great improvement patient was much stronger, walking out of doors and doing housework		61 kg.	0.28 gm.	4.6	0.08

Fifteen other cases of exophthalmic goitre gave kreatinin coefficients from 3.0 to 6.0. One of the characteristics of this disease is the muscular weakness which F. Müller by direct measurement has shown to be very great in severe cases.

Subject	Age	Weight	K ₁ -N	K ₁ -Coef.	K ₂ -N	Glucose
VII. C. B. D. Permanent biliary fistula. (This journal, xvii, p. 362.) Female. Rather weak, but walking about the house	61	55 kg.	
Greater lassitude and weakness.			0.27 gm.	4.9	..	
Walked two miles each day. Much stronger			0.22 "	4.0	..	
VIII. Ward. Chronic nephritis. Male	52	80 "	0.33 "	6.0	..	
Had been quite muscular and a hard worker, but had been in bed nearly three months.			0.36 "	4.5	..	
IX. Malkus. Chronic nephritis. Male	65	"	0.25 "	3.8	..	
Alcoholic. In bed.						
X. Kennedy. Chronic nephritis. Male	55	kg.	0.21 gm.	3.8	..	
XI. Boggs. Flat foot, obesity. Male	67	147.5	0.79 "	5.35	..	
Very inactive, but able to walk about.						
XII. Lymphatic leucæmia. Male	63		0.152 "	2.4	..	
Extremely ill.						
XIII. Mrs. A. G. Diabetes. Female. Fair condition, nutrition good, but muscles flabby and adipose excessive. Walking about. Much meat in diet.	55	77 "	0.36 "	4.65	0.25	0.5% to 2.7%
XIV. Miss E. W. W. Diabetes	50	91 "	0.43 "	4.7	0.19	3.5% to 5%
Nutrition fair, but muscles are flabby and adipose excessive. Able to walk about. Much meat in diet.						
XV. Miss A. S. Chronic nephritis. A large, athletic, and very muscular woman, but with much adipose. In bed for therapeutic reasons.	24	99.6	0.73 "	7.3	0.18	..

Subject		Age	Weight	K ₁ -N	K ₁ -Coef.	K ₂ -N
XVI.	Mrs. A. B. Normal pregnancy.	30	52 to 55	0.33	"	6.35 0.045 July 31
	Only fair muscular develop-			0.345	"	0.052 Aug. 28
	ment; slender and active.			0.362	"	0.033 Sept. 11
	During course of observa-			0.365	"	6.75 " 30
	tion patient took much exer-			0.355	"	Oct. 5
	cise (chiefly walking) out of			0.342	"	6.2 " 10
	doors, and materially in-					
	creased in strength. Normal					
	labor Oct. 18.					
	Third day <i>post partum</i> . . .			0.36	"	0.225 " 21
Subject					K ₁ -N	K ₁ -Coef. K ₂ -N.
XVII.	Mrs. A. H. Normal labor Mar. 21					
	On third day <i>post partum</i> urine contained					0.15
	" fifth " " " " "					0.23
XVIII.	Mrs. Smith. Normal labor.					
	Urine third day <i>post partum</i> contained					0.36
XIX.	Johnson. Normal labor.					
	Urine of third (?) day <i>post partum</i>					0.36
XX.	Dudd. Normal labor.					
	Urine second (?) day <i>post partum</i>					0.56
XXI.	Rupin. Typhoid fever. Male.					
	12th day of disease, temp. 102.4° to 104.8° F.			0.54	10.0	..
	43rd " " " " normal, convalescent			0.41	7.7	..
	The patient had lost but little in weight and was in					
	comparatively good physical condition.					
XXII.	Paponis. Typhoid fever. Male.					
	14th day of disease temp. 102° to 103.4° F.			0.54	9.0	0.67
	57th " " " " 100° to 103° F. Great					
	emaciation.			0.28	5.5	0.00
XXIII.	Sparks. Typhoid fever. Female.					
	July 25 temp. high			0.31	5.5 (?)	0.115
	Aug. 1 temp. normal			0.123	..	0.058
	Aug. 14 relapse			0.334	..	0.112
	Aug. 27 temp. normal			0.19	..	0.037

III. Kreatinin is not an index of the total endogenous protein katabolism. Subjects of exophthalmic goitre and others in whom the total endogenous katabolism is probably much increased may excrete very little kreatinin.

IV. Kreatinin is derived from, and its amount, expressed in milligrams per kilo body weight, is an index of, some special process of normal metabolism taking place largely, if not wholly, in the muscles. And upon the intensity of this process appears to depend the muscular efficiency of the individual.

V. The kreatinin excretion is slightly increased in acute fevers, and in this condition does not run parallel to the muscular efficiency of the individual.

VI. Kreatin is not a normal product of endogenous metabolism, and is not present in normal urines, unless the individual has taken kreatin with the food.

VII. Kreatin may be excreted by subjects of acute fevers, in the acute stages of exophthalmic goitre, in other conditions in which there is a rapid loss of muscle protein, and by women during the *post partum* resolution of the uterus.

VIII. The source of endogenous kreatin is probably the kreatin of the muscle tissues, and its appearance in urine probably indicates that muscle protein is being absorbed.

TABLE I.

KREATININ EXCRETION AND KREATININ COEFFICIENTS — NORMAL. (KREATININ COEFFICIENT = MG. KREATININ-NITROGEN PER KG. BODY WEIGHT.)

Investigator.	Subject.	Muscular development.	Remarks.	Body weight.	Kreatinin-nitrogen.	Kreatinin coefficient.
				kg.	gm.	
Folin	J. H. B.	Young and active male nurses in Hospital . . .	57.4	0.584	10.2
"	E. H. S.		66.5	0.543	8.2
"	R. L. J.		70.4	0.584	8.3
"	G. E. C.		70.0	0.658	9.4
"	M. H.		56.5	0.506	9.0
"	S. R. B.		58.0	0.587	10.1
"	O. F.	Good	Little adipose	65.2	0.587	9.0
"	"	"	Little adipose	64.1	0.554	8.6
"	"	"	Little adipose	68.3	0.584	8.5
"	H. B. H.	Good	Fat	86.0	0.584	6.8
"	"	"	Fat	86.0	0.561	6.5
"	"	"	Fat	91.0	0.673	7.4
"	E. S. A.	Fair	Lean	55.9	0.410	7.3
"	"	"	Lean	55.9	0.420	7.5
"	"	"	Lean	54.2	0.443	8.2
"	A. H.	Good	Moderately fat	70.1	0.513	7.3
"	"	"	Moderately fat	70.5	0.513	7.3
"	Dr. H.	6 ft. tall, bony but not muscular	69.5	0.502	7.2
"	Mr. B.	5 ft., 11 in. tall	75.0	0.576	7.7
"	Mr. Bu.	5 ft., 7 in. tall	64.5	0.495	7.7
"	Dr. K.	Very good	5 ft., 8 in. tall	69.5	0.685	9.9
"	Mr. W.	Fair	Lean	62.0	0.547	8.8
"	Mr. T.	Fair	5 ft., 8 in. tall	70.0	0.569	8.1
Hoogenhuyze and Verploegh	Student	71.0	0.830	11.7

TABLE I (Continued).

KREATININ EXCRETION AND KREATININ COEFFICIENTS—NORMAL. (KREATININ COEFFICIENT = MG. KREATININ-NITROGEN PER KG. BODY WEIGHT.)

Investigator.	Subject.	Muscular development.	Remarks.	Body Weight.	Kreatinin nitrogen.	Kreatinin Coefficient.
Hoogenhuyze and Verploegh	Student			kg.	gm.	
	"	80.0	0.804	10.0
	"	57.0	0.629	11.0
	"	79.0	0.822	10.4
Closson	"	63.0	0.632	10.0
	G. M. B.	Age 38, frail in physique .	61.5	0.431	7.0
	L. B. M.	Age 32, not corpulent . .	70.0	0.439	6.3
	E. H. R.	Slender	57.2	0.410	7.2
Three days excluded from average.	R. H. C.	Age 47, slender	57.0	0.331	5.8
	"	Age 47, slender	58.0	0.312	5.4
	"	Age 47, slender	59.0	0.323	5.5
	F. P. U.	Good	Age 26, not fat	65.0	0.394	6.1
Osterberg and Wolf Klercker	O. E. C.	Age 24*	62.5	0.495	7.6
	E. O.	Good	Age 40, not corpulent . .	70.0	0.465	6.65
	K. O. K.	187 cm. tall, well nourished, not fat	87.7	0.688	7.85
	O. T.	Very good	Very little adipose	68.0	0.603	8.9
Shaffer	"	"	Very little adipose	69.0	0.591	8.6
	R. A. H.	Good	Very little adipose	58.0	0.472	8.15
	R. W.	Good	Moderately fat	75.0	0.550	7.3
	P. A. S.	Fair	Slender	65.0	0.584	9.0
"	"	"	Slender	65.0	0.577	8.9
	J. T.	Fair	Slender	65.8	0.528	8.0
	J. G.	Good	Not fat	67.0	0.572	8.55
	M. S.	Good	Not fat	67.5	0.558	8.3
"	Mrs. S. T.	Good	Good	52.0	0.40	7.7

TABLE II.
HOURLY EXCRETION OF KREATININ.

Subject.	Time.	No. of hours.	Urine per hour.	Tot. N. per hour	Kreatinin per hour.	Remarks.
			c.c.	gm.	gm.	
M. S.	8 -10 A.M.	2.0	39	0.39	0.066	Net weight 62.5 kg.
"	10 -12 M.	2.0	30	0.36	0.062	
"	12 - 2 P.M.	2.0	27	0.32	0.057	
"	2 - 4 P.M.	2.0	25	0.35	0.065	
"	7 - 9.15 A.M.	2.25	50	0.64	0.068	
"	9.15-11.30 A.M.	2.25	79	0.73	0.066	
"	11.30- 1.30 P.M.	2.0	63	0.62	0.061	
"	1.30- 3.30 P.M.	2.0	67	0.62	0.062	
"	3.30- 5 P.M.	1.5	46	0.56	0.059	
"	5 - 7 P.M.	2.0	72	0.56	0.064	
"	7 - 9.30 A.M.	2.5	54	0.45	0.063	} Sleeping except for time to urinate at 3.40.
"	9.30-11.30 A.M.	2.0	67	0.51	0.065	
"	11.30- 2 P.M.	2.5	45	0.46	0.061	
"	2 - 4 P.M.	2.0	54	0.56	0.063	
"	4 - 6 P.M.	2.0	58	0.57	0.062	
"	9.15-10.50 P.M.	1.58	45	0.51	0.065	
"	10.50- 3.40 A.M.	4.83	31	0.46	0.064	
"	3.40- 5.30 A.M.	1.83	33	0.46	0.062	
"	5.30- 8 A.M.	2.5	40	0.49	0.062	
"	8 -11.30 A.M.	3.5	31	0.44	0.065	
"	11.30- 2.30 P.M.	3.0	46	0.60	0.066	} Sleeping.
"	2.30- 4.30 P.M.	2.0	49	0.61	0.061	
"	4.30-11.10 P.M.	6.67	39	0.55	0.062	
"	11.10- 8.20 A.M.	9.17	24	0.41	0.059	
"	8.20-11.30 A.M.	3.17	26	0.44	0.065	
"	11.30-12.45 P.M.	1.25	220	0.74	0.068	
"	12.45- 2.45 P.M.	2.0	60	0.69	0.061	
"	2.45- 4.15 P.M.	1.5	202	0.95	0.063	
"	4.15- 5 P.M.	0.75	930	1.11	0.063	
"	5 - 5.30 P.M.	0.5	934	1.19	0.054	
"	5.30- 6 P.M.	1.0	757	0.77	0.058	} Took 2 gm. diuretin about 4 P.M., and continued drinking much water during the afternoon.
"	6.30-12 N'T.	5.5	111	0.76	0.053	
"	12 - 9 A.M.	9.0	55	0.50	0.056	
"	9 -11.15 A.M.	2.25	60	0.45	0.062	
"	11.15-12.45 P.M.	1.5	32	0.42	0.066	
"	12.45- 4.30 P.M.	3.75	57	0.52	0.068	
"	4.30- 6 P.M.	1.5	124	0.53	0.064	
Average kreatinin 0.0625 gm. (or 1.0 mg. per kg. per hour.)						

TABLE II (Continued).

Time.	No. of hours.	Urine per hour. c.c.	Kreatinin per hour. gm.	Time.	No. of hours.	Urine per hour.	Kreatinin per hour. gm.
M. S. (one month earlier than the above). ¹				S. P. B.			
2.15- 3.15 P.M.	1.0	50	0.067	10 -11 A.M.	1.0	34	0.074 ¹¹
3.15- 4.15 P.M.	1.0	..	0.067	11 -12 M.	1.0	31	0.067
10.12-11.12 A.M.	1.0	38	0.071	12 - 1 P.M.	1.0	28	0.061
11.12-12.12 P.M.	1.0	29	0.067	1 - 2 P.M.	1.0	32	0.069
12.12- 1.12 P.M.	1.0	30	0.064	R. A. H.			
1.12- 3.12 P.M.	2.0	42	0.070	10- 11 A.M.	1.0	34	0.075 ¹²
3.12- 4.12 P.M.	1.0	53	0.071	11 - 3 P.M.	4.0	39	0.072
Average kreatinin 0.068 gm. (or 1.09 mg. per kg. per hour).				Average kreatinin 0.0735 gm. (or 1.3 mg. per kg. per hour).			
P. A. S. ²				O. T.			
Whole day	24.0	49	0.065	11 -1 P.M.	2.0	64	0.074 ¹³
7 -12 M.	5.0	34	0.068 ³	1 -3 P.M.	2.0	65	0.081
12 - 2 P.M.	2.0	96	0.068	Average kreatinin 0.0778 gm. (or 1.19 mg. per kg. per hour).			
3 - 8 P.M.	5.0	33	0.068	J. T.			
8 -11 P.M.	3.0	60	0.061	11 - 1 P.M.	2.0	70	0.049 ¹⁴
11 -12 N'T	1.0	45	0.066	1 - 3 P.M.	2.0	38	0.052
12 - 7 A.M.	7.0	36	0.060 ⁴	Whole day	24.0	66	0.059 ¹⁵
7 - 8 A.M.	1.0	28	0.063	Average kreatinin 0.0533 gm. (or 0.89 mg. per kg. per hour).			
8 -11 A.M.	3.0	87	0.070 ⁵	J. T. (eight months later, when in better physical condition).			
11 - 5 P.M.	6.0	103	0.071	1.30- 4.30 P.M.	3.0	33	0.068
P. A. S. (one month later).				I. G.			
7 - 9 A.M.	2.0	43	0.076	2 - 3 P.M.	1.0	38	0.069 ¹⁶
9 -12 M.	3.0	100	0.077	9 -11 A.M.	2.0	37	0.062
12 - 2 P.M.	2.0	231	0.074 ⁶	11 - 1.30 P.M.	2.5	34	0.070
2 - 4 P.M.	2.0	85	0.072				
4 - 6.30 P.M.	Lost				
6.30- 9 P.M.	2.5	77	0.066				
9 -10 P.M.	1.0	40	0.069				
10 - 7 A.M.	9.0	26	0.061 ⁷				
7 -10 A.M.	3.0	73	0.067				
10 -12 M.	2.0	57	0.062				
12 - 1 P.M.	1.0	230	0.074 ⁸				
1 - 2 P.M.	1.0	432	0.068				
2 - 3 P.M.	1.0	66	0.070				
3 - 5 P.M.	2.0	46	0.068				
5 - 9 P.M.	4.0	41	0.073 ⁹				
9 - 7 A.M.	10.0	40	0.070 ¹⁰				
7 -11 A.M.	4.0	55	0.071				
11 - 5 P.M.	6.0	62	0.072				
Average kreatinin 0.0686 gm. (or 1.13 mg. per kg. per hour).							

TABLE II (Continued).

Time.	No. of hours.	Urine per hour. c.c.	Kreatinin per hour. gm.	Time.	No. of hours.	Urine per hour. c.c.	Kreatinin per hour. gm.
I. G. (one month later).				10 - 6 A.M.	8.0	26	0.063
				9 - 10 A.M.	1.0	31	0.067
				Average kreatinin 0.0669 gm. (or 1.07 mg. per kg. per hour).			
11 - 1 P.M.	2.0	42	0.069	10.15-11.15 A.M.	1.0	40	0.084 ¹⁹
1 - 5 P.M.	4.0	34	0.073	11.15-12.15 P.M.	1.0	44	0.174
5 - 11 P.M.	6.0	35	0.070	12.15- 2.15 P.M.	2.0	44	0.138
11 - 7 A.M.	8.0	25	0.063 ¹⁷	2.15- 5.15 P.M.	3.0	51	0.101
7 - 10 A.M.	3.0	24	0.070	5.15-12.06 A.M.	6.85	35	0.084
10 - 1 P.M.	3.0	37	0.067	12.06- 7.20 A.M.	7.23	29	0.0725
1 - 2 P.M.	1.0	50	0.069	Total 21.08 1.9400			
2 - 4 P.M.	2.0	43	0.062				
4 - 6 P.M.	2.0	28	0.064				
6 - 10 P.M.	4.0	28	0.065				

1.94 less 1.41 (21.08 × 0.0669) = 0.53 gm. = 76% of kreatinin ingested was excreted in 21 hours.

¹ Weight same as above. ² Net weight 60.5 kg., some meat eaten. ³ A very little meat eaten at 8 A.M. ⁴ Sleeping. ⁵ Meat eaten at 8 A.M. ⁶ Drank cocoa at noon. ⁷ Sleeping. ⁸ Drank three cups of strong cocoa at 12.45. ⁹ Stewed chicken eaten at dinner. ¹⁰ Sleeping from 10 to 7. ¹¹ Net weight 56.5 kg. ¹² Net weight 65.3 kg. ¹³ Net weight 60 kg. ¹⁴ Ate some meat. ¹⁵ Net weight 62.5 kg. ¹⁷ Sleeping. ¹⁸ Sleeping. ¹⁹ Received 0.70 gm. pure kreatinin in 250 c.c. water at 10.40 A.M.

DIMINISHED MUSCULAR ACTIVITY AND PROTEIN METABOLISM.

By PHILIP A. SHAFFER.

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THERE are to be found in the literature many reports of investigations concerning the effect of increased or excessive muscular activity upon the excretion of total nitrogen, and of individual products of protein metabolism;¹ but the possible effect of greatly diminished muscular activity has received little or no attention. It has been shown repeatedly that with sufficient food a moderate increase of exercise does not lead to any considerable increase in total nitrogen excretion; but it does not necessarily follow that an abnormally diminished muscular activity may not affect some of the products of metabolism found in the urine.

Some knowledge of the effect of a low degree of activity appeared desirable as a further basis for the interpretation of metabolism results from pathological individuals, who, as a rule, are either confined in bed or take comparatively little exercise. Experiments on normal subjects were accordingly planned with a view of showing any change in the protein metabolism which might be caused by a decreased activity. "Work periods" were also included in each experiment, but the work was in no instance severe, and the results show the effect of only moderate activity. The subjects of the experiments were young men in good health.

The experiment with O. T. was divided into three periods, one which was spent almost continually in bed and at as far as possible complete rest, a second period during which the subject did his accustomed amount of work, and a third period during which he took additional but not excessive exercise. The period of accustomed work may be considered the "normal period," while the "rest

¹ Subject reviewed by MAGNUS-LEVY in VON NOORDEN'S *Handbuch der Pathologie des Stoffwechsels*, Berlin, 1906.

TABLE I.

DIETS USED IN EXPERIMENTS.

I. O. T. <i>Rest Period.</i>		Nitrogen.	Calories.
Grain rice, 60 gm.		0.86	210
Quaker puffed rice, 25 gm.		0.32	190
Quaker rolled oats, 20 gm.		0.45	
Uneeda biscuit, 60 gm.		0.87	250
Eggs (whole), 100 gm.		1.92	140
Cream (18-20 per cent fat), 225 c.c.		0.90	450
Butter, 50 gm.		0.08	400
Milk, 100 c.c.		0.51	72
Sugar, 125 gm.	513
Salt, 5 gm.
One apple \pm 150 gm.	75
		5.9 gm.	2300
II. O. T. <i>Normal Period.</i>			
Same as "Rest Period" with the addition of 50 gm. butter and 75 gm. sugar.			
Total Nitrogen, 6.0 gm.		Total Calories, 3000.	
III. O. T. <i>Work Period.</i>			
Same as "Normal Period" except 325 c.c. cream instead of 225 c.c. cream + 100 c.c. milk; 20 gm. cornstarch added.			
Total Nitrogen, 5.9 gm.		Total Calories, 3200.	
IV. O. T. <i>Non-purin.</i>			
Free mixed diet, excluding meat and meat soups. Subject ate many eggs, much milk, bread, cheese, etc.			
V. O. T. <i>Purin.</i>			
Subject ate a mixed diet, containing meat, at each meal. During periods IV and V no attempt was made to control the diets beyond the points stated.			
R. A. H. <i>First two days.</i>		Nitrogen.	Calories.
Quaker puffed rice, 56 gm.		0.78	195
Butter-thin crackers, 95 gm.		1.2	400
Butter, 45 gm.		0.07	360
Cane sugar, 90 gm.	370
Eggs (whole), 150 gm.		2.9	210
Milk, 1100 c.c.		5.8	790
Graham crackers, 45 gm.		0.5	190
		11.25	2515
R. A. H. <i>Rest of experiment.</i>			
Same as above, except 170 gm. sugar; 85 gm. puffed rice; 75 gm. butter.			
Total Nitrogen = 11.7 gm.		Total Calories, 3190.	

period " corresponds in amount of muscular activity to the condition of patients who are confined in bed and not unusually restless. The difference in the amount of muscular energy expended was obviously much greater between the rest and normal periods than between the normal and work periods.

In this experiment the rest period lasted for six days, two of which were spent wholly in bed, while on the remaining four the subject reclined quietly for a few hours each day in a Morris chair, the remainder of each day being spent in bed. The rest was so marked and prolonged as noticeably to weaken the subject, and in order to obviate any objection on this ground the rest period with R. A. H. was made only two days, which were spent wholly in bed. The experiment with R. A. H. was conducted somewhat differently in that, although the amount of muscular activity on the several days was so regulated as to make them quite different, the periods were not so sharply defined as in the other experiment.

The diets are given on page 446. That taken by O. T. was a low-protein diet, while that taken by R. A. H. contained about 11 gm. of nitrogen. Additional calories were supplied O. T. during the work periods as noted in the tables. These diets were constant except for the changes noted in the tables. After the close of the work period O. T. took a mixed high-protein non-purin diet for four days and then a diet containing much meat for six days. This change of diet was planned to obtain further data regarding the influence of meat and non-meat, and high-protein versus low-protein diets upon the composition of the urine. The urines were collected in twenty-four hour quantities.

The analytical results are given in Tables II and III. The analytical methods used are: Kjeldahl-Gunning for total nitrogen; Folin for urea, kreatinin, kreatin, and the sulphurs;² Folin-Shaffer for uric acid, and Boussingault-Shaffer for ammonia.

The conditions of these two experiments appear to me well adapted for the demonstration of any effect upon the protein metabolism (as indicated by the metabolic products of the urine), which might be caused either by a great decrease or by a marked though not excessive increase in the amount of muscular energy expended by the subjects. But on inspecting the results in the two tables we find absolutely no marked differences in the excretion of any metabolic product which can be explained by the variations in the amount

² FOLIN: *Journal of biological chemistry*, 1906, i, p. 131.

TABLE II (O. T.).

No.	Date, '06.	Volume.	Nitrogen as						Per cent of total nitrogen.				Sulphur as			
			Total.	NH ₃	Kreatinin.	Kreatin.	Uric acid.	Rest.	Urea.	NH ₃	Kreatinin.	Rest.	Total.	Inorganic.	Ethereal.	Neutral.
1	Sept. 11	1625	5.81	0.29	0.66	...	0.13	0.30	76.3	5.0	11.3	5.2	0.435	0.274	0.030	0.131
2	12	1170	5.08	0.34	0.61	...	0.12	0.39	71.2	6.8	11.9	7.7	0.469	0.297	0.045	0.127
3	13	1330	4.82	0.35	0.60	...	0.10	0.31	71.8	7.2	12.4	6.4	0.451	0.297	0.043	0.111
4	14	1015	4.38	0.38	0.59	...	0.11	0.36	67.2	8.6	13.4	8.2	0.423	0.275	0.029	0.119
5	15	1160	4.29	0.37	0.58	...	0.11	0.36	66.9	8.6	13.6	8.4	0.428	0.252	0.030	0.146
6	16	830	4.26	0.36	0.59	...	0.11	0.38	66.3	8.4	13.8	8.9	0.421	0.272	0.039	0.110
	Average ..		4.77	0.35	0.605	...	0.11	0.35	70.5	7.3	12.7	7.2	0.438	0.278	0.036	0.124
7	17	1070	4.85	0.42	0.58	...	0.12	0.41	68.6	8.7	11.9	8.5	0.422	0.267	0.031	0.124
8	18	705	4.50	0.38	0.60	...	0.09	0.42	66.9	8.4	13.4	9.3	0.427	0.271	0.047	0.109
9	19	720	4.13	0.37	0.57	...	0.10	0.42	64.6	8.9	13.8	10.2	0.404	0.259	0.046	0.099
10	20	735	4.12	0.35	0.65	...	0.11	0.42	62.9	8.4	15.7	10.2	0.420	0.274	0.041	0.105
11	21	1170	4.01	0.36	0.61	...	0.11	0.43	62.4	8.9	15.1	10.7	0.407	0.254	0.042	0.111
	Average ..		4.40	0.38	0.60	...	0.106	0.42	64.1	8.6	13.6	9.3	0.424	0.265	0.049	0.110
12	22	1260	3.78	0.36	0.65	...	0.11	0.37	60.6	9.6	17.1	9.8	0.407	0.261	0.045	0.101
13	23	1400	3.96	0.46	0.60	...	0.12	0.39	60.5	11.6	15.2	9.8	0.397	0.254	0.042	0.101
14	24	1180	3.43	0.44	0.54	...	0.12	0.39	56.6	12.9	15.6	11.4	0.398	0.252	0.039	0.107
15	25	1300	4.58	0.43	0.52	...	0.13	0.51	65.4	9.4	11.2	11.2	0.456	0.304	0.059	0.093
	Average ..		3.94	0.42	0.56	...	0.12	0.42	60.9	10.6	14.2	11.3	0.414	0.268	0.046	0.100
16	26	870	6.62	0.41	0.61	...	0.14	0.66	72.6	6.1	9.2	10.0	0.634	0.461	0.059	0.114
17	27	1610	11.82	0.69	0.56	...	0.10	0.81	81.7	5.8	4.7	6.8	0.889	0.680	0.070	0.139
18	28	1955	13.25	0.83	0.62	...	0.11	0.69	83.1	6.3	4.7	5.2	1.022	0.795	0.071	0.156
19	29	1380	12.40	0.73	0.58	...	0.10	0.94	81.1	5.9	4.7	7.5	0.962	0.750	0.070	0.142
	Average ..		11.02	0.67	0.59	...	0.11	0.775	80.5	6.0	5.3	7.2	0.877	0.646	0.068	0.138

TABLE II (O. T.).

Per cent of total S.			Total S × 100 Total N	Kreatinin.	Weight.	Mg. kreatinin per kg. body weight.	Remarks (for diets see p. 446).
Inorganic.	Ethereal.	Neutral.					
63.0	6.9	30.1	7.5	1.76	67.0		About 4 hrs. each day spent reclining in Morris chair. Rest of time in bed.
63.5	9.6	27.1	9.2	1.63			
65.9	9.8	24.3	9.3	1.62			
65.0	6.9	28.1	9.7	1.58			
58.9	7.0	34.1	10.0	1.57			
64.5	9.4	26.1	9.9	1.58	67.9		Whole time spent in bed.
63.5	8.2	28.3	9.2	1.62	24.0		
63.3	7.4	29.3	8.7	1.56	67.8		Laboratory work, but only little other activity.
63.5	11.0	25.5	9.5	1.62			
64.1	11.5	24.4	9.8	1.54			
65.3	9.7	25.0	10.2	1.75			
62.4	10.3	27.3	10.1	1.63	68.8		
62.5	11.6	25.9	9.6	1.62	23.7		Laboratory work + 10-mile walk. "Setting-up exercises" + 3½-mile walk. Walked 9½ miles + 5 hrs. hard work. Laboratory work + rapid 10-mile walk.
64.0	11.0	25.0	10.8	1.74	68.8		
64.0	10.5	25.5	10.0	1.61			
63.3	9.7	27.0	11.6	1.45			
66.6	13.0	20.4	10.0	1.39			
64.9	10.9	24.2	10.5	1.55	22.5		Normal activity.
72.5	9.5	18.0	9.6	1.64	69.1		
76.5	4.8	18.7	7.5	1.51			
77.8	7.0	15.2	7.7	1.68			
78.0	7.8	14.8	7.7	1.57			
73.8	7.8	15.7	7.95	1.60	23.2		IV. Normal Period (higher protein, non-purin diet).

TABLE II (Continued).

No.	Date, '06.	Volume.	Nitrogen as						Per cent of total nitrogen.				Sulphur as			
			Total.	NH ₃	Kreatinin.	Kreatin.	Uric acid.	Rest.	Urea.	NH ₃	Kreatinin.	Rest.	Total.	Inorganic	Ethereal.	Neutral.
20	Sept. 30	c. c. 1300	14.00	0.53	0.60	0.05	0.18	0.84	84.2	3.8	4.3	6.0	1.035	0.840	0.073	0.122
21	Oct. 1	2085	15.00	0.65	0.62	...	0.23	0.94	83.9	4.4	4.1	6.1	0.907	0.660	0.072	0.176
22	2	1780	15.20	0.65	0.65	...	0.21	0.70	85.6	4.3	4.3	4.6	1.172	0.918	0.068	0.186
23	3	1550	14.90	0.68	0.59	0.07	0.20	0.71	84.9	4.6	3.9	4.7	1.071	0.844	0.066	0.161
24	4	1860	14.88	0.54	0.65	0.07	0.20	0.85	84.6	3.7	4.3	5.7	1.077	0.863	0.073	0.141
25	5	1400	15.40	0.72	0.62	0.01	0.18	0.87	84.3	4.7	4.0	5.6	1.067	0.815	0.103	0.149
			14.90	0.63	0.62	0.03	0.20	0.82	84.3	4.2	4.2	5.8	1.055	0.823	0.076	0.156

of muscular activity. There are slight differences, to be sure, but they are in no case great enough to justify the conclusion that the amount of muscular activity is responsible. At the bottom of each table will be found the averages of the three periods; an inspection of these averages fully bears out the above statement. There are, however, some points of considerable interest which must be referred to.

A much discussed question in connection with the effect of muscular work has been its effect on kreatinin excretion, and it was largely my interest in this question which suggested these experiments.

In my experiments upon a patient with a permanent biliary fistula³ and in many other yet unpublished experiments on diseased individuals, I have found a greatly decreased kreatinin excretion, which was increased when the patients improved sufficiently to take more exercise. The conclusion was therefore tentatively held that the diminished kreatinin excretion was a result of diminished muscular activity. In the meanwhile there appeared the paper of Hoogenhuyze and Verploegh⁴ in which they conclude that with

³ SHAFFER: This journal, 1906, xvii, p. 362.

⁴ HOOGENHUYZE and VERPLOEGH: Zeitschrift für physiologische Chemie, 1905, xli, p. 415. This paper contains a review of the literature.

TABLE II (Continued).

Per cent of total S.			Total S X 100 Total N	Kreatinin.	Weight.	Mg. kreatinin per kg. body weight.	Remarks (for diets see p. 446).
Inorganic.	Ethereal.	Neutral.					
81.1	7.1	11.8	7.4	1.61	...		} Normal activity V. Normal Period (high protein, purin diet).
73.0	7.6	19.4	6.0	1.67	...		
78.3	5.9	15.8	7.7	1.74	...		
78.6	6.4	15.0	7.2	1.58	...		
80.3	6.6	13.1	7.2	1.74	...		
76.5	9.5	14.0	6.9	1.67	...		
78.0	7.2	14.8	7.1	1.67	...	24.2	

sufficient food muscular activity has no effect upon kreatinin excretion. However, in view of my results from pathological individuals it seemed to me possible that their negative conclusions could be explained by there being in their experiments perhaps no great difference between the amount of energy expended on the control days and that expended on the work days.⁵ Whether or not this is a just criticism, it was decided to further test the question.

For reasons stated I fully expected to find a decreased kreatinin excretion in the subjects of my experiments during the rest periods. But the results show nothing of the sort. I therefore agree with the conclusion of Hoogenhuyze and Verploegh, as well as of certain earlier investigators, that muscular activity with adequate food has *per se* no effect on the excretion of kreatinin, which in these experiments is remarkably constant and quite independent of both the amount of food protein (Folin) and of the amount of muscular activity.

⁵ The observers experimented upon themselves. They did each day their accustomed amount of laboratory work and on stated days took bicycle rides and gymnastic exercises. On account of the common inclination to remain quiet after marked fatigue it seems not unlikely that after their vigorous exercises the subjects may have rested for some hours, thus tending to reduce the difference in muscular activity between the control days and the work days.

TABLE III: (R. A. H.).

Twenty-four hours ending 8 A. M.	Volume.	Nitrogen.					Per cent of total nitrogen.			Sulphur as			
		Total.	Ammonia.	Kreatinin.	Uric acid.	Rest.	Urea.	Ammonia.	Rest.	Total.	Inorganic.	Ethereal.	Neutral.
Sept. '06.													
22	860	8.57	0.214	0.54	0.135	0.39	85.0	2.5	4.5	0.611	0.459	0.059	0.093
23	600	8.05	0.267	0.46	0.143	0.56	82.2	3.3	6.9	0.671	0.520	0.046	0.105
24	740	9.30	0.285	0.49	0.128	0.54	84.5	3.1	5.7	0.775	0.611	0.049	0.115
25	690	9.72	0.276	0.45	0.127	0.46	86.6	2.8	4.7	0.734	0.576	0.054	0.104
26	795	11.04	0.42	0.47	0.103	0.56	85.9	3.8	5.1	0.796	0.623	0.057	0.116
27	710	9.63	0.38	0.46	0.127	0.42	85.5	3.9	4.4
28	690	10.46	0.40	0.48	0.147	0.61	84.4	3.8	5.9
29	710	9.34	0.41	0.48	0.123	0.52	83.6	4.4	5.5	0.695	0.529	0.051	0.115
30	1020	9.27	0.38	0.46	0.108	0.55	83.5	4.1	5.95	0.664	0.493	0.061	0.110
Oct. 1	670	8.51	0.44	0.47	0.124	0.56	81.7	5.2	6.6	0.668	0.507	0.045	0.116
Av. of 30 and 1....		8.89	0.41	0.465	0.116	0.555	82.7	4.6	6.25	0.666	0.500	0.063	0.113
Av. 23, 27, 28, 29 ..		9.37	0.386	0.47	0.135	0.53	84.0	4.1	5.6	0.683	0.524	0.049	0.110
Av. 22, 24, 25, 26 ..		9.66	0.30	0.487	0.123	0.487	85.5	3.1	5.0	0.729	0.567	0.055	0.107

TABLE III. (R. A. H.).

Per cent of total S.				Weight.	Kreatinin.	Mg. kreatinin per kg. body weight.	Remarks.
Inorganic.	Ethereal.	Neutral.	Total S \times 100 Total N.				
75.0	9.7	15.3	7.1	60.0	1.45	24.2	Laboratory work. Walked 9 miles. 1 hr. work out-doors.
77.5	6.9	15.6	8.3	...	1.24	20.6	Laboratory work. Walked 1 mile. Little activity.
78.8	6.3	14.9	8.3	...	1.32	22.0	Walked 7 miles. 2 hrs. work out-doors. Diet increased (see p. 446).
78.5	7.4	14.1	7.6	...	1.21	20.2	Walked 2½ miles. 7 hrs. work out-doors.
78.2	7.2	14.6	7.2	57.8	1.28	22.2	Laboratory work. Walked 5 miles.
...	1.24	21.5	In bed 11 hrs. Very little activity.
...	1.28	22.2	Laboratory work, but little other activity.
76.1	7.3	16.5	7.4	...	1.30	22.5	Laboratory work, but little other activity.
74.3	9.2	16.6	7.2	58.0	1.23	21.2	Whole 24 hrs. in bed.
76.0	6.7	17.3	7.8	...	1.26	21.8	Whole 24 hrs. in bed.
75.0	7.95	17.0	7.5	58.0	1.245	21.5	Whole time spent at complete rest in bed.
76.7	7.2	16.1	7.3	± 58.0	1.265	21.7	Laboratory work. Somewhat less than normal activity.
77.7	7.6	14.7	7.5	± 58.5	1.315	22.15	Extra work. More than customary exercise.

It is worthy of notice how slight was the increase of kreatinin during the period with meat diet (Table II). The average for six days is only 0.05 gm. (1.67 gm. kreatinin) more than on the low-protein non-meat diet (1.62 gm.). According to Folin⁶ and Klercker⁷ it is only the kreatinin (*i. e.*, not the kreatin) in the food (meat) which increases the kreatinin of the urine, and therefore the effect of the character of the food upon kreatinin excretion will depend upon the amount of kreatinin contained in the food eaten. Kreatin was not present in the urine of the subjects of these experiments, except in small amounts during the meat diet of O. T.

In Table II (O. T.) the total nitrogen decreased with increased activity, while exactly the opposite is found with R. A. H. It is unlikely that the amount of activity is in either case responsible. R. A. H. was losing weight during the first part of the experiment, while O. T. gained in weight during the whole time of the low-protein diet, and was undoubtedly storing protein. The figures regarding urea and ammonia again confirm Folin's statements, that the percentage of total nitrogen represented by urea decreases with decrease of total nitrogen, and that the absolute amount of ammonia decreases while the ammonia percentage increases, with decrease of total nitrogen. This is shown very strikingly in Table II. Neither urea nor ammonia was affected by change in the amount of muscular activity.

The excretion of uric acid is, according to these experiments, wholly unaffected by a decreased muscular activity.⁸ Regarding the relation of uric acid to food, Folin⁹ has found it to be slightly increased with increase of total nitrogen. In Table II the uric acid nitrogen (with non-purin diet) is not higher with 11 gm. total nitrogen than it was with 4 gm. total nitrogen.

⁶ FOLIN: *Festschrift für Hammarsten*, 1906, iii, Upsala.

⁷ KLERCKER: *Biochemische Zeitschrift*, 1907, iii, p. 45.

⁸ CATHCART, KENNAWAY, and LEATHES: *Quarterly journal of medicine*, 1908, i, p. 416, Oxford, show that a marked increase of muscular activity causes, during the work, a decreased excretion of uric acid, which is followed, soon after the work has ceased, by an increased excretion. Within the narrower limits of the amount of work, as in my experiments, the excretion of uric acid is not affected.

⁹ FOLIN: *This journal*, 1905, xiii, p. 86. FOLIN's conclusion is somewhat contrary to that of BURIAN and SCHUR (*Archiv für die gesammte Physiologie*, 1900-1903, lxxx, lxxxvii, and xciv), and of SIVEN (*Skandinavisk Archiv für Physiologie*, 1901, xi), who found the endogenous excretion of uric acid to be constant.

There is no evidence that the undetermined nitrogen is affected in any way by the change in muscular activity. The differences are relatively slight and almost, if not quite, within the limit of error.

The figures in Table II confirm Folin's "law" concerning undetermined nitrogen.

The total sulphur runs parallel with the total nitrogen, the inorganic sulphur parallel with urea, and the ethereal sulphur practically parallel with ammonia. No one of the above is affected by muscular activity. In the case of neutral sulphur there is, in each experiment, a decrease with increase of muscular activity; but I do not conclude that the neutral sulphur always decreases with an increase of activity. Further experiments are necessary to decide this point. The neutral sulphur increases materially with increase of total sulphur, but not in proportion (Folin,¹⁰ Table II).

The results of these experiments support the belief that with sufficient food either an increase or a decrease of muscular activity within physiological limits has *per se* no effect upon the protein metabolism as indicated by the nitrogen and sulphur partitions in the urine. We cannot, of course, believe that a long-continued diminished activity would not cause a change in the composition of the urine, because the intensity of metabolic processes in a muscle atrophied from disuse is certainly less than in a healthy muscle; but such a change in the composition of the urine should be considered not the direct result of decreased activity, but the result of a pathological condition, which, it may be, was brought about by a diminished activity. Exercise is necessary for health, but the amount of muscular energy expended in a given day (provided the amount is not excessive for the particular subject) does not appear to affect any of the nitrogenous substances of the urine excreted on that or following days.

I am indebted to two of my colleagues, Drs. Oscar Teague and R. A. Hatcher, who kindly consented to be the subjects of these experiments.

¹⁰ SHAFFER: This journal, 1906, xvii, p. 375.

PROTEIN METABOLISM IN CYSTINURIA.¹

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This paper deals with the results of an investigation of the metabolism in two cases of cystinuria. It is unnecessary to give an outline at the beginning, either of the history or the peculiar features of the anomaly, for an analytical critique of the literature is given by Moreigne, Simon, and more recently by Neuberg in v. Noorden's *Handbuch der Pathologie des Stoffwechsels*.

One of our cases, the first to be examined, was observed over a period of eight and one-half months, during which time we have conducted five separate metabolism experiments, using different constant diets and have made complete analyses of the urine on eighty-one days. During these experiments we have administered to the patient various substances in an attempt to clear up certain specific points. As these points can be more conveniently referred to when the analytical results are presented, they will be discussed apart from the general consideration of the metabolism in this affection.

The second case is probably unique in the history of cystinuria, and presented an unusual opportunity to examine the relation

¹We wish to thank Drs. C. E. Nammack, Alexander Lambert and Cyrus Strong, the attending physicians of the Fourth Medical Division of Bellevue Hospital for their courtesy in permitting us the examination of the first patient. Our thanks are especially due to Drs. C. K. Stillman, R. R. Ryan, G. B. Emory, F. W. Rice and H. C. Thacher, house physicians in this division, for their unfailing willingness to give us every possible assistance during the experimental work. For the second case, we are indebted to Prof. L. A. Stimson and the house surgeons of the New York Hospital for great assistance during a very difficult investigation.

of bile sulphur to urine cystin in cystinuria. The patient was a woman suffering from an impaction of the ductus communis choledochus, and had been operated on for gallstones. Calculi had been removed from the gall-bladder. During the time she was under observation there was a fistulous opening, through which all the bile could be collected. Unfortunately the patient proved refractory; it was quite impossible to induce her to take a constant diet, and we were also unable to collect accurately twenty-four hour specimens of urine. It is certain that much of the urine on some of the days was passed with the stools. The case was also most remarkable in that during the course of the examination the cystinuria disappeared. While this fact has been noted by other observers, it has never occurred that the transition took place during the course of the examination. It is to our very great regret that the records of this very singular case are so incomplete.

The experiments on the two cases will be discussed separately.

The plan of our experiments was essentially the same as that adopted by Alsberg and Folin. The patient was placed on a fairly constant diet, and complete analyses of the urine made, embracing the following fractions. Total nitrogen (Kjeldahl); urea (Folin); ammonia (Folin and Boussingault-Shaffer); creatinin and creatin (Folin); uric acid (Folin-Shaffer); rest nitrogen by difference; total sulfur, inorganic sulfates, ethereal sulfur and neutral sulfur (Folin). The analytical results with the notes regarding the specific diets will be found in the tables.

CASE I. The extract from the case history is as follows:

J. S. male, age 44. Nativity, Sweden. Admitted to Bellevue Hospital, October 19, 1906.

Present History: Has used beer and whisky freely for years. Appetite always poor. Bowels move daily. Has had gonorrhea twice in the last two years.

Previous illness: Eight years ago patient was ill for four or five months. Says illness was identical with present. Denies any other illness.

Present illness: Onset two weeks ago. Swelling of both ankles, accompanied by signs of inflammation, redness and local heat. Two days later both ankles became painful. Swelling of left hand. Both knees have become somewhat painful and stiff.

Complaints: Pain, stiffness and swelling. Temperature, 100.4° F.; pulse, 120; respirations, 24.

Physical examination: Adult male, fairly well nourished. Not acutely ill. Some pain when ankles and hand are moved. Slightly anemic. Heart normal. No fibrosis of arteries. Lungs, abdomen, liver and spleen normal. Lymph nodes not enlarged. Joints, very slight swelling and tenderness.

Diagnosis: Rheumatic fever, subacute.

The points which have been investigated with this patient are the following:

(1) The quantitative variation in the composition of the urine with diets containing varying amounts of protein.

(2) What proportion of the food protein sulfur is excreted as cystin?

(3) The degree of tolerance of the cystinuric for free cystin and cystein, when given by the mouth.

(4) The degree of tolerance of the cystinuric for cystein and cystin when administered subcutaneously.

(5) The degree of tolerance of the cystinuric for other amino-acids.

(6) A study of the time relations of carbon, sulfur and nitrogen, and some of the fractions of nitrogen and sulfur groups after the administration of protein.

THE QUANTITATIVE COMPOSITION OF CYSTINURIA URINES.

With the exception of a single set of analyses, by one of us and Marriott, Alsberg and Folin are the only investigators who have made a fairly complete analysis of cystinuria urines, using satisfactory methods. This these authors did each day during the whole of their experiments on the effect of diet upon the excretion of cystin. We also have made complete analyses each day, and have therefore a means of comparing the metabolism of our case, in so far as it is represented by analyses of the urine, with that of Alsberg and Folin, and with normal individuals on the same diets. At the foot of Tables VII and VIII are given averages, representing normal urines (for the particular diet and amount of total nitrogen), and also the averages of Alsberg and Folin from their case of cystinuria. A comparison of these averages with the averages from our own case shows the amount and character of the deviation from the normal, and from the Alsberg-Folin patient.

In the first experiment (Table VI) in which a high protein, milk-egg diet was given, the averages of our control days compare remarkably well with those obtained by Alsberg and Folin. The only important differences are our higher rest nitrogen and higher neutral sulfur, in spite of the fact that our patient was 12 kilogrammes lighter in weight. It is worthy of note that the average percentage of neutral sulfur was 10 per cent higher.

Compared with the normal averages, we find the same variations as those noted by Alsberg and Folin. The ammonia nitrogen is about half the normal and the rest nitrogen is very high. The ratio between total sulfur and total nitrogen is quite within the normal limits; but the neutral sulfur, including the cystin sulfur is more than four times greater than normal, making the relative percentage 37.0 per cent instead of the normal 7.3 per cent. The ratio between total nitrogen and neutral sulfur is 3.1 as compared with 0.6, the normal ratio for this amount of total nitrogen.

On the low protein diet, the abnormalities become much less pronounced. (See control averages, Tables VII and VIII.) When the patient catabolized only little food protein the composition of the urine was more nearly, but not quite normal. On such a diet, the ammonia is practically normal. The rest nitrogen, while still high is decidedly nearer the normal figures, in one experiment (Table VII) being 0.58 gram, and in the other (Table VIII) 0.79 gram, as compared with Alsberg and Folin's 0.69 and 0.86 gram, and the normal 0.40 and 0.42 gram.

The absolute amount of rest nitrogen in this case as in the normal depends upon the amount of protein catabolized. As an instance of this fact, the reader is referred to the results of the experiment (Table VIII) in which 50 grams of casein were administered while the patient was on a low protein diet. The rest nitrogen was increased from about 0.8 gram to 1.14 gram, the average of two days after the casein ingestion. The ratios between the sulfur and nitrogen are normal (compared with Shaffer's averages from a normal individual on the same rice-cream diet); but the ratio of neutral sulfur to total nitrogen is again high, being in one experiment 5.5 per cent as against the normal 1.6 to 2.5 per cent.

The marked anomalies in our case of cystinuria as well as in

that of Alsberg and Folin are therefore, (a) a very high neutral sulfur, (b) remarkably low ammonia nitrogen, and (c) high rest nitrogen.

The increase of neutral sulfur is certainly in part and possibly wholly due to the sulfur of the cystin. The nitrogen of the cystin is included in the "rest nitrogen" fraction, but even assuming that all the excess of neutral sulfur is due to the cystin, *that amount of cystin is not sufficient to explain the high rest nitrogen.* For instance, on the high protein diet, the excess of neutral sulphur is $(0.45 - 0.10) = 0.35$ gram, which is equivalent to 0.15 gram of cystin-nitrogen. The excess of rest nitrogen is more than 1.0 gram ($\pm 1.8 - 0.6$). *There must therefore be one or more stable nitrogenous substances outside of those examined for and other than cystin in the urine in this condition.* In the examination of the experimental sulfur anomaly produced by poisoning with brombenzol, one of us with Marriott has come to a similar conclusion. We have so far not been able to make a systematic search for these substances, and it is useless to conjecture their identity. Simon, and Alsberg and Folin suggest that diamins are present in a larger percentage of cases than is at present supposed. We have on two occasions attempted to find diamins in the urine of this patient by the Baumann-Udransky method, but without success. Moreover, diamins could not possibly be the cause of the high undetermined nitrogen, as these substances are volatile with steam, and so would be found in the urea distillate. Some experiments which we have performed show that pentamethylenediamin when added to urine does not come over in appreciable quantity either by the Folin-air method, or by the Boussingault-Shaffer method for ammonia, so that diamins would not be included in the ammonia fraction. Whatever the substances are composing the rest nitrogen in cases of cystinuria, it is evident that their amount depends upon the weight of food protein catabolized; the less the total nitrogen, the nearer to normal values does the rest nitrogen become. The low ammonia excretion is, as Alsberg and Folin suggest, probably due to the smaller amount of protein sulfur excreted in the form of sulfates.

THE EFFECT OF THE AMOUNT OF PROTEIN INGESTED ON THE
AMOUNT OF CYSTIN EXCRETED.

A fundamental problem towards the solution of which many of our experiments have been directed is the source of the cystin which is excreted in the urine. The questions which we wished to decide were:

(1) Is the urine cystin derived directly from food protein? If so, what percentage of the food protein is excreted as cystin?

(2) Is all of the urine cystin derived directly from food protein? In other words, is the urine cystin wholly exogenous?

To answer these questions demands that we be able to determine the amount of cystin excreted. So far as we are aware, there is no accurate method for the determination of cystin in the urine. The procedure usually adopted is to weigh the cystin which has been precipitated from the urine by means of acetic acid. In some cases the urine is evaporated in a vacuum before precipitation. That precipitation by acids is not satisfactory is shown by Mester's experiment, in which he obtained but 4.0 per cent of the cystin added to urine. We also have failed to obtain even approximately complete recovery of cystin by acidification with acetic acid, and other workers have met with the same difficulty. Evaporation in a vacuum doubtless increases the yield of cystin but the results are by no means quantitative. Substances which are present in the urine hold the cystin in solution. The other methods which have been employed, such as precipitation with benzoyl chlorid, α -naphthyl isocyanate, and β -naphthalin sulfochlorid, are all unsatisfactory from a quantitative point of view, for with these reagents other substances are precipitated and an accurate separation of the cystin is impossible.¹

In our experiments, the amount of cystin excreted is deduced from the amount of neutral sulfur in the urine. In such a calculation we must assume that the whole of the excess of neutral

¹ Since this work was finished, Gaskell has suggested that the precipitation by means of acetic acid be made in the presence of acetone. It is quite possible that this method may be more satisfactory than any heretofore employed. We have not had an opportunity to give it a sufficient trial to be able to speak of its accuracy.

sulfur over the amount found in normal individuals is due to cystin. Mester, and Alsberg and Folin have also made use of this assumption. That this is not necessarily correct must be admitted, for it is by no means certain that the neutral sulfur output in a cystinuric urine, exclusive of cystin is the same as that in a normal individual; or, that the sulfur containing compounds, over and above the normal neutral sulfur are exclusively cystin.

As however the accurate determination of cystin is beset with so many difficulties, one is almost forced in a long series of experiments to deduce the amount of cystin from the excess of neutral sulfur. We believe, with Alsberg and Folin, that the calculation of the amount of cystin from the neutral sulfur is more nearly satisfactory than the results of any other method now available.

The relation of diet to the elimination of cystin was investigated very early by Pletzer and Toel, who found that foods containing a low amount of nitrogen increased the cystin output. This was confirmed by Ebstein who fed his patients beans. Cantani went so far as to prescribe a meat diet in the affection as a means of decreasing the amount of cystin. Bartels on the other hand was unable to confirm these results. Mester in 1890 reinvestigated the whole matter in a more systematic manner, and fed his patient on various diets. Unfortunately for the present purpose the amounts of nitrogen excreted in the urine are not given. He did however estimate the amount of total sulfur and total sulfate-sulfur excreted in the twenty-four hours, and from this calculated the amount of neutral sulfur.

Accepting the total sulfur output as an index of the total amount of nitrogen catabolized, the various diets given by Mester do not seem to have varied very considerably in their nitrogen content. Thus, for example, on a meat diet 1.28 grams of sulfur were eliminated, while on what is termed a carbohydrate diet, 1.07 gram of sulfur were excreted. From this one may conclude that the change in the diet, in so far as the nitrogen content was concerned, was not very radical. The highest amount of neutral sulfur was excreted on a vegetable diet, the lowest on a mixed diet. One must conclude from these observations that the cystin excretion (neutral sulfur) is greatest

on a vegetarian diet. Thus Mester apparently confirmed the statements of the older investigators.

The next detailed report of the behavior of a cystinuric with different diets is given by Alsberg and Folin. These investigators used two very different diets. One contained about 19.0 grams of nitrogen as protein, and the other consisting chiefly of starch and cream contained less than 1.0 gram of nitrogen. The averages of their results, which have already been mentioned, will be found in our tables. Their results show that, with the great decrease in food protein, there was a marked decrease in neutral sulfur excretion. On the diet containing very little protein, the neutral sulfur, in spite of being greatly diminished in absolute amount, was relatively very much more prominent than when more protein was catabolized. And, assuming that the excess neutral sulfur represents cystin, they conclude that the amount of cystin depends primarily upon the amount of protein catabolized. In view of the fact that "cystin crystals were found in the urine at the end of a thirteen day period with diet containing practically no protein," and in view of the relatively high neutral sulfur from low protein diets, they further conclude that part of the urine cystin is derived directly from the tissues.

In a recent paper Thiele has taken occasion to controvert these views. Thiele's experiments on this point are not in the least satisfactory. Not only has he used the precipitation of cystin with acetic acid, with Abderhalden's modification of preliminary evaporation *in vacuo* but he has attempted to decide the question using periods of only one day for each of the two diets. As is shown in Alsberg and Folin's experiments, the decrease of neutral sulfur (and therefore, presumably cystin) is not very well marked on the first day of a low protein diet. It is quite certain that no decisions on involved questions as these can be reached with experiments of such a type.

The following averages taken from our own tables show the relation between the cystin excretion (calculated from the excess of neutral sulfur) and the amount of total nitrogen and total sulfur catabolized.

TABLE I.

Control averages from table.	Total nitrogen of urine.	Total sulfur.	Neutral sulfur.	Normal neutral sulphur.	Cystin S. (= Neut. S. - normal neut. S.).	Cystin S per cent of Total S.	100 Cystin S. Total N.
I High protein diet.	14.63	1.24	0.45	0.10	0.35	28.2	2.4
II Low protein diet.	3.53	0.318	0.195	0.07	0.125	39.3	3.5
III Low protein diet.	4.15	0.378	0.199	0.07	0.129	34.1	3.1
<i>7 mo. later:</i>							
IV Fairly high protein diet.	11.35	0.895	0.307	0.10	0.207	23.2	1.8
IV Low protein diet.	4.81	0.43	0.26	0.07	0.19	44.1	3.9
Alsberg and Folin.	14.84	1.17	0.328	0.10	0.228	19.5	1.5
Alsberg and Folin.	4.62	0.344	0.192	0.07	0.122	35.4	2.6
Alsberg and Folin.	5.19	0.388	0.226	0.07	0.156	40.2	3.0

See also Table IV, July 1, when 12 eggs were added to the diet.

Accepting the method of determining the cystin, the figures show conclusively that the amount of cystin excreted by this case, and by Alsberg and Folin's case depends, in the first instance, on the amount of sulfur catabolized. A further argument in favor of the truth of this statement is the fact that the urines from the high protein diet deposited heavy cystin sediments, while the urines from the low protein diets contained a much smaller amount of a similar sediment. According to our results, about 25 per cent of the total sulfur was excreted as cystin when a high protein diet was fed, while on a low protein diet the cystin represented 35 to 40 per cent of the total sulfur.

Since the amount of cystin excreted depends upon the amount of food protein, we agree with Alsberg and Folin that the cystin is, in part, derived directly from the food, and is therefore of exogenous origin. Because of the relative increase of the cystin sulfur with decrease of exogenous metabolism, we must also conclude with these investigators that a part of the cystin is derived from the tissue catabolism, and is therefore of endogenous origin.

Were the cystin wholly exogenous, we should expect the

per cent of total sulfur excreted as cystin to be decreased rather than increased when the products of exogenous metabolism are greatly lowered by decreasing the protein intake.

The whole problem of exogenous and endogenous metabolism, as far as the results which can be drawn from experiments in starvation and on low protein diet is in our estimation complicated by the recent considerations of Freund and his co-workers. These investigators have directed attention to the condition which probably occurs during abstention from food. Using the older observations on the secretion of the succus entericus, they have shown that a much larger amount of protein is thrown into the alimentary canal by this means than is usually presumed to be the case. This protein is probably catabolized in exactly the same way as is food protein. It therefore must be very difficult, when one speculates on a product such as endogenous cystin, to differentiate that cystin which is actually produced without the intervention of the alimentary canal from that produced by the cleavage of protein thrown into the alimentary canal by means of the succus entericus. It is for this reason, perhaps, that the variations in starvation and in low protein diet do not appear as sharply as one might be led to expect. A type of digestion and resorption is apparently proceeding during abstention from food, which is in every way similar to that produced by the assimilation of food. In fact, it would seem from Freund's point of view that it would be quite impossible to suppress any metabolic process completely which normally has as its basis the catabolism of food protein in the intestinal canal.

THE DEGREE OF TOLERANCE OF THE CYSTINURIC FOR FREE CYSTIN
AND CYSTEIN WHEN GIVEN BY THE MOUTH.

The normal human organism, as has been repeatedly shown, completely oxidizes to sulfuric acid the sulfur of cystin when this substance is given by the mouth.

In an investigation of the metabolism of a cystinuric by Loewy and Neuberg in 1904 it was shown that protein cystin given by the mouth to their patient was almost quantitatively eliminated as such, and no oxidation of sulfur to sulfuric acid took place. Alsberg and Folin, on the other hand, were unable to confirm this

interesting observation on the patient which they examined, but found that cystin when given by the mouth to a cystinuric was as completely oxidized as by the normal subject. Thiele has recently confirmed this result. It is impossible to harmonize these wholly contradictory results, except on the assumption that the metabolic processes in the two cases were very different, in spite of the fact that the two individuals excreted cystin.

In the hope of throwing some light on this situation, we have determined with our case his tolerance for both cystin and cystein when given by mouth. In all, seven experiments on this point were performed. From the results of the urine analyses, one can readily determine whether the sulfur of the ingested cystin was excreted unoxidized (Loewy and Neuberg) or was oxidized in the body, and appeared in the urine as inorganic sulfates (Alsberg and Folin). The significant results of these experiments are brought together in Table II.

It is not necessary to describe the experiments at length. (The details will be found in Table II and in the complete tables at the end of this paper.) The results lead to the conclusion that cystin, prepared from hair, cystein, prepared from protein cystin, cystin isolated from the patient's urine—that cystin which he had previously failed to oxidize—when fed by the mouth, in so far as it was absorbed by the intestine, was oxidized and the sulfur was excreted in the form of sulfates. The very slight increase in neutral sulfur after the feeding of urine cystin, cystein, and the large amount of cystin (10 grams) may possibly indicate a *tendency* towards intolerance, but it is not safe to draw this conclusion from such small differences. The increase in inorganic sulfates after each cystin or cystein feeding is very marked. In this respect also, our results are in agreement with those of Alsberg and Folin, and are contradictory to those of Loewy and Neuberg.

This is certainly a very interesting situation. As has been shown in a preceding section, food protein leads in these individuals to the excretion of unoxidized cystin; but when food protein is hydrolyzed outside the body, and the isolated cystin given them, this cystin does not pass through the body as such, but appears in the urine in the form of sulfates.

Cystinuria

TABLE II.

	Total nitrogen.	Total sulfur.	INORGANIC SULFUR.		NEUTRAL SULFUR.		100 Total S	
			gm.	Per cent of total S.	gm.	Per cent of total S.	Total N	Neutral S
<i>High protein diet.</i>	Sum of 2 days preceding cystin.	2.72	1.68	61.7	0.907	33.3	8.4	2.8
	Sum of 2 days following ingestion of 5 gm. cystin.	3.09	2.12	68.5	0.864	28.0	10.2	2.85
	Increase following cystin by mouth.	+0.37	+0.44		-0.043			
	Control average of 14 days \times 2 for 2 day average.	7.06	0.178	28.0	0.39	61.0	9.0	5.5
<i>Low protein diet.</i>	Sum of 2 days after feeding 5 gm. of cystin from hair.	6.66	0.498	55.5	0.34	37.9	13.5	5.1
	Increase following hair cystin by mouth.	-0.40	+0.320		-0.06			
	Sum of 2 days after feeding 1.72 gm. urine cystin.	6.98	0.451	48.5	0.427	46.0	13.3	6.1
	Increase following urine cystin by mouth.	-0.08	+0.273		+0.037			

<i>Low protein diet.</i>	Control average + 2 for 2 day average.	8.30	0.756	0.282	37.3	0.398	52.7	9.1	4.8
	Sum of 2 days after feeding 3 gm. hair cystin.	7.84	1.127	0.666	59.2	0.383	34.0	14.4	4.9
	Increase following hair cystin by mouth.	-0.46	-0.371	+0.384		-0.015			
	Sum of 2 days after feeding 3 gm. cystin.	8.43	1.184	0.666	56.1	0.453	38.2	14.1	5.4
	Increase following cystin by mouth.	+0.13	+0.428	+0.384		+0.055			
<i>High protein diet.</i>	Day preceding cystin	12.53	0.973	0.597	61.4	0.304	31.4	7.8	2.4
	Day following 10 gm. hair cystin by mouth.	12.50	2.163	1.711	79.1	0.368	17.0	17.3	2.9
	Increase following cystin by mouth.	-0.03	+1.190	+1.114		+0.064			
<i>Low protein diet.</i>	Control average for 2 days.	9.62	0.86	0.26	30.2	0.52	60.5	8.9	5.4
	Sum of 2 days following 3.7 gm. hair-cystin by mouth.	11.40	1.44	0.86	75.5	0.52	36.0	12.6	4.6
	Increase following cystin by mouth.	+1.78	+0.58	+0.50		0.00			

The marked difference in the behavior of cystin as such and the sulfur containing group of the protein molecule is worthy of further close investigation. A similar state of affairs to that seen in cystinuria is observed in animals poisoned by brombenzol. While the ingestion of meat leads to a greatly increased output in the amount of neutral sulfur and undetermined nitrogen, as *p*-bromphenylmercapturic acid, the ingestion of cystin, or of the acid itself in animals under the influence of brombenzol leads to no distinct increase in neutral sulfur.

A sufficient number of experiments with the behavior of cystin in the cystinuric are now on record to show, that with the single exception of Loewy and Neuberg's case, the capacity of the cystinuric individual for catabolizing cystin when given by the mouth is nearly complete. One can therefore be fairly positive that in the digestion and absorption of protein, hydrolysis does not take place as far as cystin, but what hydrolysis does occur, is to a degree in which the cystin group is protected from oxidation.

Loewy and Neuberg with exactly the opposite results concerning the fate of ingested cystin results were able to draw a similar conclusion, and have ingeniously suggested a method which would determine to what extent hydrolysis had occurred before absorption, by determining the point at which after giving a definite hydrolytic product of protein oxidation took place. Their suggestion appears to us well worthy of experimental trial.

THE DEGREE OF TOLERANCE OF THE CYSTINURIC FOR CYSTIN AND CYSTEIN WHEN ADMINISTERED SUBCUTANEOUSLY.

Three experiments on the fate of cystein and cystin when introduced into the organism outside the alimentary canal were performed. The results are given in the following table:

TABLE III.

	Total nitrogen.	Total sulfur.	INORGANIC SULFUR.		NEUTRAL SULFUR.		Total S.	Total N.
			gm.	Per cent of total S.	gm.	Per cent of total S.	100	100
<i>High protein diet</i>	Average of 2 days before injection (November 9 and 10)	13.26	1.00	59.4	0.594	33.8	7.5	2.5
	± 1 gm. cystin injected subcutaneously (November 11)	15.60	1.29	53.1	0.685	41.9	8.3	3.5
	Increase following injection		0.29	0.091	0.091	0.202		
<i>Low protein diet</i>	Day before injection: (November 28) ± 1 gm. cystin injected subcutaneously (November 29)	3.93	0.292	24.3	0.071	60.0	9.6	5.7
	Increase following injection	3.34	0.353	27.8	0.098	62.3	10.6	6.6
			0.061	0.027	0.027	0.045		
<i>Low protein diet</i>	Control average	3.53	0.318	28.0	0.089	61.0	9.0	5.5
	× 3 for 3 day average	10.59	0.954	28.0	0.267	61.0	9.0	5.5
	4 gm. cystin injected subcutaneously 3 days following injection: December 10	4.07	0.747	45.8	0.342	49.5	18.4	9.1
	December 11	2.81	0.353	23.2	0.082	71.0	12.5	8.9
	December 12	4.71	0.423	26.5	0.112	65.0	12.0	7.9
	Sum of 3 days following injection	11.59	1.523	35.2	0.536	58.8	13.2	7.7
	Increase following injection	1.00	0.569	0.269	0.269	0.310		

On the high protein diet, about 1.0 gram of cystin dissolved in sodium carbonate was injected into the loose tissue beneath the breast. The total sulfur was increased 0.29 gram; the inorganic sulfur 0.09 gram, and the neutral sulfur was increased 0.20 gram.

Three weeks later, about 1.0 gram of cystein was dissolved in 0.85 per cent sodium chlorid, and was similarly injected. The increase in the total sulfur was very slight, but the greater part of this increase was found in the neutral sulfur.¹

The third and last experiment on this point was the injection of 4.0 grams of cystein dissolved in 0.85 per cent sodium chlorid. The injection took place beneath the scapula of the left side.² The effects of this injection seem to be shown in the three succeeding days, and for this reason the results of the three days are given in the table. The total sulfur was increased 0.57 gram, of which 0.27 gram falls on the inorganic sulfur, and 0.31 gram falls on the neutral sulfur. From these results we can merely conclude that the subcutaneous injection of cystin and cystein lead to a marked increase in the non-oxidized sulfur, and to a smaller increase in the inorganic sulfur. Our results do not prove that the increase of neutral sulfur is due to cystin, but we believe this to be the case for the following reasons. Blum injected cystin subcutaneously into normal dogs. The sulfur was oxidized to sulfuric acid. Injected rapidly into the peripheral circulation, cystin was, in part, excreted unchanged. Injected into the mesenteric veins, no cystin could be recovered from the urine. This appeared to Blum to denote that the slowing of the circulation of cystin when perfused through the liver gave time for the substance to be oxidized to sulfates, while in the greater circulation the cystin reached the kidneys rapidly,

¹The amount of cystein given in this injection is uncertain. The cystein was partly oxidized to cystin, and the crystals formed blocked the syringe. An unknown amount of the solution was lost.

²There was almost an immediate rise in temperature of the patient, and a very decided amount of toxic disturbance, as shown by dizziness, headache, and great malaise. The patient was put to bed. Ten days later an abscess developed. It would appear that the injection of such a large amount of cystein may be associated with distinct toxic symptoms. We were unwilling to repeat the experiment.

and was there excreted. By flooding the organism extra-intestinally as was done in our case, it is more than probable that some of the cystin reached the kidney and was excreted.

THE DEGREE OF TOLERANCE OF THE CYSTINURIC FOR SULFUR-FREE AMINO-ACIDS.

An important part of the work of Loewy and Neuberger was a study of the tolerance of their patient for amino-acids not containing sulfur. The data which have accumulated seem to show that in many cases the metabolic anomaly in cystinuria does not consist solely in the excretion of cystin, but associated with the thioamino acid in the urine may be leucin, tyrosin, and possibly tryptophan. That other amino-acids than cystin might also be present would appear very probable, yet the detection of these acids has been made in the minority of cases only. In the case of Loewy and Neuberger no other amino-acids than cystin were found in the urine. Following however the oral administration of tyrosin, leucin and aspartic acid, these substances appeared almost quantitatively in the urine. In addition, the diamino-acids, arginin and lysin were not completely metabolized as they are in the normal individual, but were excreted as the diamins, putrescin and cadaverin, although the patient under ordinary conditions did not eliminate these substances. On the other hand, Alsberg and Folin did not find any lack of tolerance on the part of their patient for aspartic acid and tyrosin. The nitrogen of these substances was excreted quantitatively as urea. Simon, Garrod and Hurthley, and Thiele were likewise unable to detect any intolerance in their several patients for amino-acids.

The results of our experiments on this point are not altogether clear. We gave our patient tyrosin, asparagin and glycocoll. The amount of tyrosin given (1.0 gram) was too small to show any effect, but the urine of that day did not show any Millon reaction. On a low protein diet we gave the patient 10.0 grams of asparagin. There was a slight increase in the rest nitrogen (0.25 gram) and a considerable increase in the urea nitrogen (0.7 gram). Again, on a low protein diet we gave the patient

20.0 grams of glycocoll. The total nitrogen of the succeeding days was increased 3.33 grams ($5.64 + 5.33 + 4.80 = 18.78 - 3 \times 4.15 = 3.33$). The urea nitrogen was increased 2.35 grams ($3.65 + 3.56 + 3.18 = 10.39 - 3 \times 2.68$) and the rest nitrogen 0.73 ($1.29 + 0.92 + 0.89 = 3.10 - 3 \times 0.79$). In each of the latter two experiments, the greater part of the nitrogen (about 70 per cent) fed as amino-acids was excreted as urea. There was therefore in our case no marked intolerance for amino-acids. It is impossible to be perfectly sure as yet as to the exact fate of amino-acids especially in man, for few experiments have been made where the amino-acids have been followed by a technique such as we have used and it would appear that experiments of this kind are urgently needed to settle the tolerance of normal individuals for the different products of the hydrolysis of proteins. It appears to us that the method of judging of their excretion by means of an analysis of the urine as we have done gives a more accurate method of determining the fate of the acids than the use of reagents for the amino-acids which give at the best very incomplete precipitations. Using for comparison the results of the various investigators who have followed the fate of the amino-acids in the human organism, it would appear that the cystinuric does not differ essentially from the normal subject in his capacity for converting these substances into urea.

THE INFLUENCE OF SODIUM CHOLATE ON THE METABOLISM OF THE CYSTINURIC.

Some years ago v. Bergmann observed that in dogs with a biliary fistula, the feeding of cystin caused no increase in the biliary sulfur; but by feeding cholic acid, especially after the ingestion of cystin, there was a notable increase of sulfur in the bile. In order to ascertain whether the increase in the neutral sulfur of the urine was due to a lack of cholic acid in the organism, so that the taurin synthesis, which undoubtedly arises from the cystin complex, was inhibited, Simon and Campbell fed cholic acid to a cystinuric. No definite change was observed in the type of the sulfur metabolism. From this they concluded, that either no combination of cholic acid with cystin

takes place as Blum had suggested, or that cystin is not changed to taurin.

In controlling Simon and Campbell's result, we gave our patient, during the time in which he was on a low protein diet (December 1, 1906) 3 grams of sodium cholate in four divided doses during twelve hours. The effect of this procedure does not agree with what Alsberg obtained with cats. The total nitrogen elimination fell from 3.61 grams to 3.03 grams. No corresponding effect was noted on the excretion of total sulfur.

It will be noted that the rest nitrogen and neutral sulfur do not entirely agree. While the relative value of the latter fell decidedly, there was a slight rise in the relation of the former to the total nitrogen. This might be explained by the occurrence of oxidation of the cystin sulfur leaving the amino group intact in the form of an amino lactic acid. As will be shown in the second case which we are about to report, the administration of cystin to a cystinuric does actually increase the amount of sulfur in the bile. In order to reconcile this observation with that of v. Bergmann who found no increase in the sulfur of the bile in normal dogs after feeding cystin, it will be necessary to perform experiments on the feeding of cystin to dogs with biliary fistulas who are under the influence of brombenzol. If v. Bergmann's experiments hold good for all normal animals, and the increase in the sulfur constituents of the bile occurs in all cystinurics, one has here a fundamental difference in metabolism which has heretofore not been pointed out.

THE EXCRETION OF CREATININ AND CREATIN IN THE CYSTINURIC

The recent experiments of Folin and of Klercker have demonstrated, contrary to what has been the accepted idea, that there is no direct relationship between the creatin taken in with the food and the amount of creatinin in the urine. When pure creatin was fed, a part was indeed excreted as creatin, but the amount of creatinin was not affected. When however creatinin was fed, at least 80 per cent of this substance passed through the body unchanged. While it was *a priori* unlikely that a cystinuric would behave differently from the normal subject

with respect to these substances, the opportunity of observing a patient on a diet which would display any anomaly was not to be neglected. Accordingly, on a low protein diet (Table II), 5.0 grams of creatin (= 1.6 gram of nitrogen) were given by the mouth. In the following 20 hours, 1.44 gram (= 0.46 gram of nitrogen) were eliminated. The creatinin excretion was not affected.

Four days later, 5.0 grams of creatinin were given by the mouth. In the following twenty hours, 3.2 grams of this substance, in addition to the usual amount of creatinin which was to be found in his urine were excreted on this day. This equals an excretion of unchanged creatinin for the first twenty hours of 64 per cent. With the normal subject, Folin found 80 per cent of the creatinin administered excreted unchanged in the urine. Folin's normal subject, receiving about 1.0 gram of nitrogen in his food, excreted from 0.0 to 20 per cent of the ingested creatin and about 75 per cent of the ingested creatinin. Our cystinuric patient, on a diet containing about 5 grams of nitrogen excreted 64 per cent of the ingested creatinin and 29 per cent of the ingested creatin. Sufficient data are not at hand to determine whether or not the excretion of exogenous creatinin by this patient is normal.

THE TIME RELATIONS OF CARBON, NITROGEN AND SULFUR IN CYSTINURIA.

As one of us has stated in a previous paper in this *Journal*, the amount of work which has been done on the time relations of the individual components of the urine, outside of the carbon and nitrogen is exceedingly small. When one comes to examine the literature of pathological conditions, practically no information is available as to the effect of abnormal conditions on the time relationships of excretion. This is especially the case when one comes to deal with relationships involving hourly examination.

Our object in performing this experiment was to ascertain if any one of the anomalies which are found in cystinuria, viz: low ammonia, low urea, high undetermined nitrogen or high neutral sulfur made their appearance at any specific time after

the ingestion of protein. In the case of the last two fractions one should be able to observe whether the suspected non-sulfur amino-acid fraction made its appearance in advance of the cystin. It is very unfortunate that no experiments on normal individuals are available with which to compare this experiment. Outside of the experiments of v. Feder with dogs on hourly relations of sulfur, nitrogen and phosphorus, Tschlenoff and Slosse and Hamäläinen and Helme who fractionated the sulfur compounds of the urine we have no standards for comparison. The experiments of the Swedish investigators were unfortunately done with periods of twenty-four hours, and are therefore not comparable with ours.

The plan of the experiment was as follows: While the patient was on a non-nitrogenous, starch-cream diet (for some time), 50 grams of casein (Hammarsten) of Kahlbaum was administered in one dose at 9 a. m. Every four hours following the administration, the urine was collected separately and analyzed. Owing to the number of determinations which we wished to make, this seemed to be the most frequent collection which we could employ. The methods used for analysis were the same as those employed in the rest of the work. The urines were analyzed for carbon by evaporating a measured volume of the urine at a low temperature in a weighed dish and burning a fair sample of the weighed residue. Owing to the small quantity of urine available, the determination of uric acid was not performed. This component is included in the undetermined nitrogen.

Results. During the first four hours all the component parts of the urine were at a low level. During the second period, eight hours after the administration of the casein, there was a sudden rise in the excretion of all the constituents. Two of the components reached their maximum at this time (see Fig. 1). The remaining fractions reached a maximum at the third period. It will be seen that there is a much greater delay in the elimination of sulfur and nitrogen than was found by Feder in his experiments with dogs after a large amount of protein. On the other hand, the results are in agreement with what was found by one of us with Marriott with dogs under the influence of brombenzol. In administering a large quantity of meat to a dog under the influence of this poison, the maximum elimination of the nitrogen

did not take place till the end of the second four-hour period, although other fractions of the urine, notably the amino-acid nitrogen, and creatinin nitrogen reached a maximum during the first four hours.

It would be unwise to generalize from these two experiments, so widely different in character, but the similarity is sufficiently striking to be pointed out. The urines of the fourth period, that is to say, sixteen hours after the ingestion of the protein contained least of all the fractions examined. From that time on, there was a secondary rise. To what this secondary rise was due is difficult to say. It was not seen in the brombenzol experiment but is indicated in the curves of Feder's experiments with normal dogs. It is possible that in the course of digestion a preliminary breaking down and resorption of some of the products of hydrolysis takes place. This produces the first and highest rise in the curves of elimination. This is followed by a second apex due perhaps to a final resorption and catabolism of the more difficultly resorbable products.

What is well marked is the early elimination of ammonia nitrogen. From this it would appear that early deamidation and resorption of ammonia takes place. This is accompanied by the setting free of molecules containing a high percentage of carbon. It is to the elimination of these substances that the early carbon maximum is due. From Feder's experiments, it would appear that the sulfur is the component which is most quickly excreted. This is certainly not seen in these experiments. While the sulfur elimination rises during the first four hours, the maximum is not reached until eight hours have passed.

It is instructive also to consider the relations of some of the constituents to one another from the point of view of hourly excretion. In this way one may obtain information regarding the proportion of nitrogen and sulfur which are excreted as the various fractions. As with the absolute excretion, most of the nitrogen excreted as ammonia occurs in the first four hours. Three of the relation curves show a marked similarity. These are the relation of total sulfur to total nitrogen, rest nitrogen to total nitrogen and neutral sulfur to total sulfur. The first curve is more exaggerated than the other two, but the trend is in absolute agreement. It is possible to infer from this that the

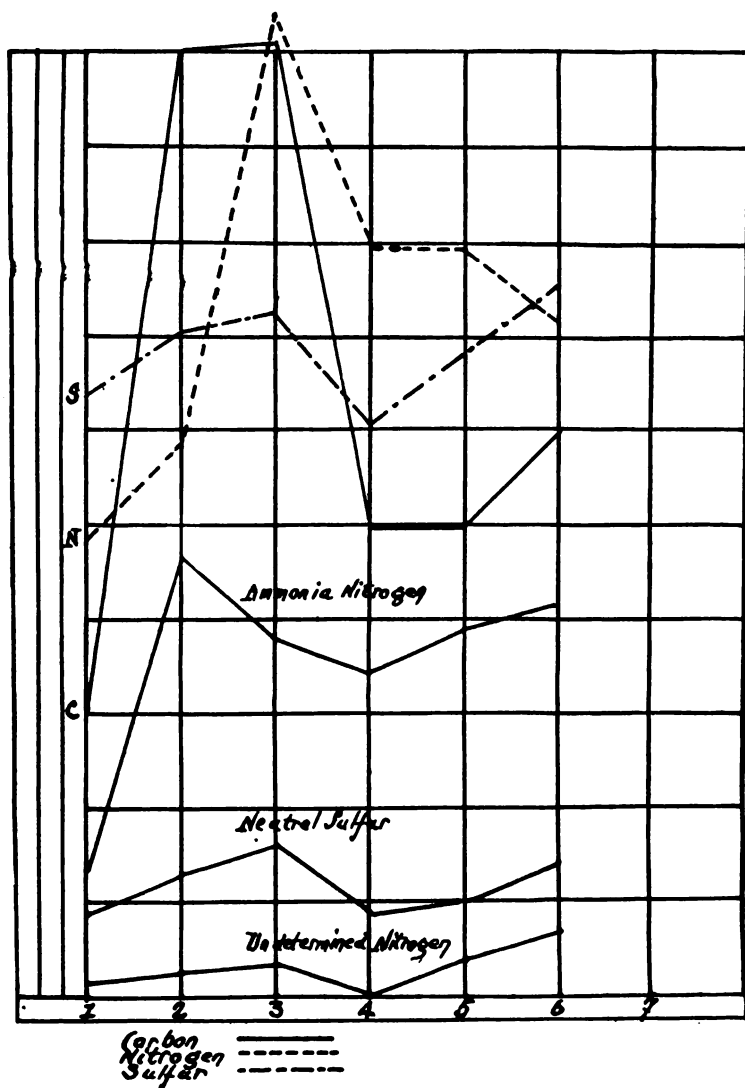


FIG. 1

TIME RELATIONS OF EXCRETION IN CYSTINURIA. (See Table VIII.)

processes giving rise to rest nitrogen and neutral sulfur are either identical or run an absolutely parallel course. Curiously enough, the course which the neutral sulfur to total sulfur takes is not quite in agreement with the three above mentioned, for instead of being the exact antipode, as might be expected, the trend of the curve is parallel to the others in the fourth period.

CASE II. This case is of such exceptional rarity, that despite the incompleteness of the results with regard to the collections of the urines and the information regarding the diet, we have decided to publish the analytical findings in full. (Table X.)

The history is as follows:

S. M. admitted to hospital September 11, 1906; discharged September 16, 1906. Readmitted November 19, discharged December 26.

Diagnosis: Typhoid fever with cholecystitis. Family history negative. Present history, none. Habits, none.

Present condition: General malaise. Physical examination: Large frame. Adipose tissue excessive. Pulse regular in size and force. Heart normal. Lungs normal. Abdomen soft, adipose tissue felt in masses. Liver percusses from fifth space to costal margin, edge not felt. A slight tenderness over the lower half of the abdomen. Spleen, percusses, enlarged. Edge felt one finger breadth below the costal margin. Skin: Few rows of spots over abdomen and chest. History omitted to September 28. At this date, jaundice of the conjunctivæ was much less. Abdomen not distended, but held rather tightly on right side. No pain or tenderness. Enlargement of the liver still persists. Note of today says cystin (?) crystals in the urine. She insists upon going home. A hard mass is felt in the right upper quadrant. It consists of rounded globules. The urinary examination showed cystin crystals September 22, 26, 28. Sp. gr. varies 1010 to 1030. The temperature varied from time of admission from 104.4° to 98.6° F. Blood examination. Leucocytes October 1, 11,000; October 4, 13,000; October 7, 10,000. Differential count gives, October 13, 13,200 leucocytes = polynuclear, 63 per cent, lymphocytes 37 per cent, hemoglobin = 58 per cent. There was no blood in the stools.

Readmission. An operation for cholecystotomy was performed November 20. The gall-bladder was evacuated of about 500 cc. of an extremely thin fluid and about 50 gallstones of various sizes scooped out. The largest about the size of a hazel nut. December 1, all drains removed, a rubber tube inserted into the opening and packed with gauze. This drainage tube was connected with a bottle. On December 5, a special diet of rice, cream, milk, crackers, butter, salt and fruit was given.

During this period, the pulse varied from 92 to 128. Respirations, 20 to 28, temperature 100° to 103°. The stools were clay colored, and free

from hydrobilirubin, except on December 5, 6, 7, 8, 9, 17, when there was a trace. On November 27, a special diet of eggs, milk and crackers was given.

The points of interest in connection with this case are:

(1) The effect of the diversion of bile on the excretion of cystin in a cystinuric.

One of us some time ago made a detailed examination of the protein metabolism in a case of complete biliary fistula. The urine was examined during periods of high and low protein diet which were free from purins. As a result of this study, it did not appear that the neutral sulfur was derived from the taurin of the bile, except in those cases associated with icterus where resorption of bile from the biliary passages took place. The neutral sulfur of the urine was not lower than is normally the case on the same quality and quantity of food. This first patient is of interest as furnishing us with standards for comparison in the present examination.

In comparing the results of the analysis of the urine and the bile of the two cases, it does not seem that outside of the effect of cystinuria itself on the urine, any marked change was to be found as a result of the diversion of the bile stream. With regard to the composition of the bile, some differences are to be observed, but they are not altogether pronounced. For a somewhat similar nitrogen and sulfur output the following table of comparison will serve to indicate the differences. The results have been chosen from the two cases as far as possible to be in accord.

At the beginning of our experiment with a biliary fistula in a cystinuric (23,11) there are very marked differences both between the nitrogen and sulfur partitions of the cystinuria and the normal case. In the former the gross urea relation is much lower than the rest nitrogen higher; the alkaline sulfate sulfur ratio is remarkably low, and the neutral sulfur high. On comparing the ratios which were obtained towards the end of the experiment when the cystinuria phenomena had practically ceased, the ratios both for sulfur and for nitrogen are in fairly close agreement with the simple case of biliary fistula.

The excretion of nitrogen and sulfur by the bile is higher in the case of cystinuria than in the normal case, but the relations of the two components are nearly the same. The changes are

TABLE IV.
Cystinuria case with biliary fistula. (See Table X.)

URINE.										BILE.					
Date.	Vol- ume.	Total nitro- gen.	Gross urea N.	NH ₃ N	Creat- inin N.	Uric acid N.	Rest N.	Total sulfur.	Alkaline S.	Ethereal S.	Neutral S.	100 parts of 1000	Nitro- gen.	Sulfur.	100 S N.
			per cent.	per cent.	per cent.	per cent.	per cent.		per cent.	per cent.	per cent.				
Nov. 23	500	6.60	4.58 69.4	0.25 3.7	0.26 3.9	0.16 2.4	1.60 24.3	0.426	0.081 19.0	0.013 3.2	0.332 77.8	6.5			
Dec. 8	455	4.33	3.33 76.9	0.33 7.6	0.21 4.9	0.02 0.5	0.77 17.7	0.287	0.177 61.8	0.026 9.0	0.084 29.2	6.6	0.55	0.092	16.7
Dec. 12	720	6.81	5.90 86.6	0.65 9.6	0.23 3.5	0.04 0.6	0.58 8.4	0.414	0.218 52.7	0.082 19.7	0.114 27.6	6.1	0.60	0.072	12.0

Biliary fistula C. B. D. (Shaffer).

Oct. 31	740	7.56	6.59 87.2	0.77 10.2	0.26 3.4	0.14 1.9	0.57 8.5	0.42	0.26 61.2	0.08 18.1	0.09 20.7	5.5	0.225	0.035	15.5
Nov. 5	560	4.56	3.81 83.3	0.94 20.6	0.20 4.4	0.08 1.8	0.47 10.3	0.34	0.22 61.8	0.05 14.7	0.08 23.5	7.6	0.41	0.075	21.9

therefore of a quantitative nature only, and do not apparently involve any qualitative alteration.

(2) The disappearance of the cystinuria during the course of the examination.

In order to follow the disappearance of the cystinuria during the course of the examination, recourse must be had not merely to a qualitative test for the cystin as such, but to the relation of the various components of the sulfur. As many of the urines are incomplete, in order to form some idea of the amount of nitrogen which was being eliminated, it is necessary to take as a criterion for nearly complete urines, the day on which the highest amount of creatinin was eliminated. This was on October 23, on which day 0.26 gram of creatinin nitrogen were excreted. Placing all urines which have a creatinin nitrogen content between 0.18 and 0.23 as approximately complete, those days which can be relied upon to a certain extent are December 8, 9, 11, 12, 15, 18, 19, 20, 21, 23. The total nitrogen excretion varied from 3.60 to 7.23 grams. The lower amount is on the border line for urines on a diet sufficient in caloric value, but containing small amounts of protein. In judging the urines it seems wise to use as a standard the lowest values given by Folin on this diet.

In a review of the highest values for neutral sulfur and the lowest values for alkaline sulfur given by Folin it is found that the lowest relation of alkaline sulfur to total sulfur was 54.6 per cent for a normal subject, a patient however giving a value of 42.6 per cent. The highest relative value for neutral sulfur was 37.4 per cent. At the beginning of the present series of analyses we have alkaline sulfate sulfur forming but 19 per cent of the total sulfur, and the neutral sulfur 77.8 per cent. Immediately after this the ratio of neutral sulfur to total sulfur began to fall, and on December 8, two weeks after the first examination the ratios, compared with Folin's figures, had become altogether normal. In comparing the sulfur partition with that of the woman with a biliary fistula, it will be seen that at no time did the ratio of neutral sulfur to total sulfur in the latter case exceed 24 per cent.

The results obtained from an examination of the nitrogen partition are not quite so clear. While on the first day of the

examination the highest relative value for undetermined nitrogen to total nitrogen—24.3 per cent—was obtained, the value throughout the whole experiment was high, much above that found in normal individuals. There was however a distinct tendency to fall. One may suspect that while the cystin excretion decreased during the experiment a greater proportion of non-deamidated substances were still being eliminated than is normally the case. It has been shown on a number of occasions that the excretion of cystin is associated with the elimination of sulfur free amino acids. It may be that while the cystin output fell a disturbance leading to the excretion of these substances still continued.

During the course of this work we have had an opportunity to examine single twenty-four hour cases of cystinuria urines and in Table V are given the results of these analyses.¹

SUMMARY.

(1) *The anomalies in the metabolism of cystinuria.*

These consist in low ammonia, high undetermined nitrogen and high neutral sulfur. The high ammonia, as Alsberg and Folin suggest is due to the small elimination of sulfur in the form of sulfates. In some cases, as in the second, and in the case of Marriott and Wolf, high ammonia is indeed seen. In any event, the sum of ammonia plus urea nitrogen is below that found in normal individuals on the same diet. The high undetermined nitrogen is in part due to cystin, and is in part due to other amino-acids; for the ratio of amino-acid nitrogen to neutral sulfur is much above that found in normal subjects. Cystinuria is probably never a simple anomaly in which the cystin complex is the only part of the protein molecule which is affected. Owing to the difficulty of their separation, it is impossible to say what are the other fractions which are concerned in the increase in the undetermined nitrogen. The high neutral sulfur is probably due entirely to cystin. No evidence has been obtained that the

¹ For the first of these urines we are indebted to the kindness of Dr. Abrams of Providence, R. I., and for the second to Mr. Hoffmann, of Cornell Medical College,

TABLE V.
Urines of two other cases of cystinuria.

Volume Sp. gr. 1.0—	Total N. Reac- tion.	Gross urea N.	NH ₂ N.	Urea N.	Creatinin N.	Creatin N.	Uric acid N.	Rest N.	Total S 100 Tot. S Tot. N.	Tot. sul- fate S.	Alkaline S.	Ethereal S.	Neutral S.
		per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.		per cent.	per cent.	per cent.	per cent.
<i>Case I*</i>													
2100	14.8	11.6	0.15	11.4	0.57	0.0	0.210	2.46	1.135	0.758	0.663	0.096	0.377
17	alk.	78.2	1.0	77.2	3.8	0.0	1.4	16.6	7.6	67.3	58.8	8.5	33.5
<i>Case II†</i>													
1055	7.47	5.95	0.298	5.65	0.36	0.084	0.106	0.971	0.894	0.626	0.585	0.041	0.268
13	ac.	79.7	4.0	75.7	4.8	1.1	1.4	13.0	11.9	70.1	65.4	4.7	30.0

*3.62 gm. of crude β -naphthalin sulfochloride compounds obtained from the 24 hour urine.

†Faint Millon reaction in this urine.

fluctuations which are found in the neutral sulfur following changes in diet are due to any substances other than cystin. In other cases of cystinuria, viz: those in which amino-acids have been found in the urine, it would be highly important to determine whether the administration of protein increases the output of undetermined nitrogen above that which is found in normal subjects, and whether the tolerance for the free acids when given by the mouth is the same as in the normal. Were the tolerance normal for an individual free acid and the increase after protein feeding great, one would have an indication that these acids were also absorbed from the intestine in the form of higher complexes.

(2) *The origin of the cystin of the urine.*

The cystin in high protein feeding is largely of exogenous origin; but a part is probably not derived directly from food-protein. To what extent strictly endogenous processes play a part in its formation is impossible to say. With the excretion of the intestinal juice, catabolism of protein proceeds in the same way as after the ingestion of protein. The form in which the cystin sulfur is absorbed during the digestion of protein demands much further investigation, especially with improved methods for actually determining the cystin output in the urine, with methods which will leave no doubt that the increase in the neutral sulfur is actually cystin.

That the cystin sulfur of the protein molecule is not absorbed as cystin seems to be shown beyond reasonable doubt. This has been shown in our experiments in two ways. First: the ingestion of protein in the cystinuric leads to a greatly increased output of neutral sulfur and presumably of cystin in the urine. Cystin and cystein, administered by the mouth are completely catabolized to sulfates. Second: Cystin and cystein administered subcutaneously are in part oxidized, and in part excreted as neutral sulfur. One must therefore assume that the cystin group is not absorbed as such, but passes through the intestinal wall in combination with other amino acid groups as polypeptids or thioalbumoses. The hydrolysis of these compounds takes place perhaps in the liver.

(3) *The degree of tolerance of the cystinuric for cystin.*

The results which we have obtained are in substantial agreement with those of Alsberg and Folin, in showing undiminished

tolerance for cystin on the part of the cystinuric when this substance was given by the mouth. They are contradictory to the findings of Loewy and Neuberg. In each of the three sets of observations the results have been so decisive that it is necessary to formulate some hypothesis to reconcile them.

Alsberg and Folin believe that but one type of cystinuria exists. Neuberg classifies the affection into three divisions. While it seems probable that the latter arbitrary division will not be found adequate to completely cover the condition, cystinuria does in reality exist with different degrees of severity. It has been shown by us that cystinuria (Case II) may practically cease within a short time, and this has also been observed by others under less favorable conditions. Had it been possible to follow the tolerance of this patient for proteins, one would probably have found a point at which a definite amount of protein was without influence on the elimination of cystin. If this be so, it is reasonable to infer that in the severer grades of cystinuria, one may have those in which diminished tolerance for cystin or other amino-acids may be present. The fact also that diamins are found in certain cases and are assuredly not present in others leads one to believe that in this one also has perhaps a fundamental point of difference in the types of cases. It may again be emphasized that as yet no one has suggested what the point of connection of these substances in the urine has with the main anomalies of cystinuria.

(4) *The effect of subcutaneous injections of cystin and cystein on the cystinuric.*

Unfortunately we have no experiments on normal individuals with which to compare our results. The investigations of Blum on dogs and rabbits enable us to form some notion of the fate of cystin when introduced by paths other than the intestinal tract. He has shown that the rapidity of injection and whether the substance be introduced into the peripheral circulation or into a mesenteric vein determines to a large extent the course which its elimination takes. Blum attributes the occurrence of complete oxidation after mesenteric injection of cystin to the slowing of the blood current in the terminal vessels of the liver. From our results it is impossible to say whether an excessive excretion of cystin, over that which would be found in the normal

individual, as a result of the injection of cystin, took place. What is shown however is the capacity of this patient to oxidize a part of this cystin when introduced subcutaneously. It is probable in the flooding of the organism with such large quantities of cystin and cystein, part of the substances reached the liver and were there oxidized. Whether part of the oxidation took place in the muscles and other organs is impossible to decide. No experiments on the feeding of cystin to animals with an Eck fistula have so far been described. We hope to give the details of some experiments on this point in a later communication.

(5) *The fate of sulfur-free amino-acids.*

The results which we have obtained agree with those of Alsberg and Folin. Glycocoll, asparagin and tyrosin when administered by the mouth did not perceptibly increase the output of undetermined nitrogen. The nitrogen of these substances was almost quantitatively catabolized to urea.

(6) *The fate of creatinin and creatin in the cystinuric.*

The excretion of creatinin and creatin after administration by the mouth did not differ in any essential particular from what has been observed in normal individuals.

(7) *The effect of sodium cholate on the elimination of cystin.*

v. Bergmann showed in animals with a biliary fistula that the elimination of sulfur in the bile was independent of the ingestion of cystin, while sodium cholate increased the amount of sulfur in this fluid. The subsequent administration of cystin increased the output of sulfur above the amount of which was provoked by the ingestion of the cholate. It was therefore concluded that the secretion of sulfur in the bile was dependent on the reserve of cholic acid in the body. Simon gave cholic acid to a cystinuric in the hope of decreasing the amount of cystin excreted, but was unsuccessful. Our results with sodium cholate are in agreement with the last observation. No noticeable effect on the output of neutral sulfur was indicated after the treatment.

(8) *The time relations after protein feeding.*

The experiment performed was of such a nature that as yet we have no standards in the normal subject with which to compare it. It shows however that the maximum of nitrogen took place later than that of carbon, and that the former was coinci-

dent with that of sulfur. The maximum for ammonia nitrogen was early. The trends of the curves showing the elimination of neutral sulfur and undetermined nitrogen were alike.

(9) *Cystinuria and its relations to the bile.*

We have previously referred to the work of v. Bergmann and Simon who have investigated certain points in connection with cystin and the excretion of sulfur by the bile (section 6). In a comparison of a case of biliary fistula in an otherwise normal subject which has been reported by one of us (Shaffer) with a similar condition in a cystinuric, we have been unable to note any distinct difference in the composition of the bile which may be referred to the cystinuria, or in the composition of the urine which may be referred to the diversion of bile from the intestine. In all the analyses of the bile made on this case, the relation of sulfur to nitrogen varied only between the limits which were also found in the case of simple biliary fistula. One point however was observed which may distinguish the cystinuric from the normal individual: the administration of cystin by the mouth appeared to raise the amount of sulfur excreted by the bile. The relation of sulfur to nitrogen during this proceeding was altered. Control experiments with a normal subject with a biliary fistula with the administration of cystin must be made to decide whether the above mentioned behavior is characteristic for cystinuria.

(10) *The disappearance of the cystinuria during the examination.*

In their second paper on cystinuria, Loewy and Neuberg have referred to the cases of this anomaly which have ceased with lapse of time. The second case which we report shows this very plainly. While the distribution of the sulfur components of the urine returned almost to normal ratios, the nitrogen partition was still characterized by a very high undetermined nitrogen on some days. That the diversion of the bile was not responsible for this condition is deduced by the fact that in Shaffer's case the biliary fistula did not perceptibly change the ratios of the nitrogen constituents of the urine. We are inclined to believe that while the anomaly in metabolism which consists in the elimination of cystin disappeared during the time the patient was under observation, the high relative undetermined nitrogen indicates that a greater proportion of substances which come under the head of amino-acids as still being excreted. It is

difficult to estimate what connection, if any, existed between the diversion of the bile stream from the intestine and the disappearance of the cystin from the urine. As one of us (Shaffer) has found, the neutral sulfur of the urine in normal subjects probably does not have its source in the taurin of the bile. While it is unlikely that the establishment of a biliary fistula had any effect on the cessation of the cystinuria, the coincidence of the change in the metabolism appearing so soon after the operation is sufficiently striking to throw doubt on such a conclusion.

LITERATURE.

- Abderhalden and Schittenhelm: *Zeitschr. f. physiol. Chem.*, xlv, p. 468, 1905.
 Alsberg: *Journ. Med. Research*, xiii, p. 105, 1905.
 Alsberg and Folin: *Amer. Journ. of Physiol.* xiv, p. 54, 1905.
 Bartels: cited by Mester.
 v. Bergmann: Hofmeister's *Beiträge*, iv, p. 132, 1904.
 Blum: Hofmeister's *Beiträge*, v, p. 1, 1903.
 Cantani: cited by Mester.
 Folin: *Amer. Journ. of Physiol.*, xiii, p. 64 and 73, 1905. Hammarsten's *Festschrift*, 1906.
 Freund: *Zeitschr. f. exp. Pathol. u. Therap.*, iii, p. 633, 1906; iv, p. 1, 1907.
 Garrod and Hurthley: *Journ. of Physiol.*, xxxiv, p. 217, 1906.
 Gaskell, *Journ. of Physiol.*, xxxvi, p. 142, 1907.
 Klercker: Hofmeister's *Beiträge*, viii, p. 59, 1906.
 Loewy and Neuberg: *Versammlung deutsch. Naturf. Aertzte*, 1903; *Zeitschr. f. physiol. Chem.*, xliii, p. 338, 1904; *Biochem. Zeitschr.* ii, p. 213, 1906.
 Marriott and Wolf: *Amer. Journ. Med. Sci.*, February, 1906; *Biochem. Zeitschr.*, vii, p. 213, 1907.
 Mester: *Zeitschr. f. physiol. Chem.*, xiv, p. 109, 1890.
 Moreigne: *Compt. rend. soc. biol.*, 1, p. 1097, 1898; *Arch. med. exp.* xi, p. 254, 1899.
 Pletzer: cited by Mester.
 Shaffer: *Amer. Journ. of Physiol.*, xvii, p. 362, 1906.
 Simon: *Amer. Journ. Med. Sci.*, xix, p. 48, 1900; *Zeitschr. f. physiol. Chem.*, xlv, p. 357, 1905.
 Simon and Campbell: Hofmeister's *Beiträge*, v, p. 401, 1904.
 Slosse: *Trav. Inst. Solvay*.
 Thiele: *Journ. of Physiol.*, xxxv, p. 68, 1907.
 Tschlenoff: *Korrespondenzblatt Schw. Artze.*, xxvi, p. 348, 1896.

100 Neutral S. Total N.	Weight, kg.	REMARKS.
4.5		Low protein diet. 720 grams boiled rice, 120 cc. cream, about 200 gm. sugar.
6.6		
5.1		
5.7		
6.6		
5.8		± 1 gm. cystein given hypodermically.
5.8		
6.8		
7.1		
* 6.7*		
4.6		10 gm. asparagin (= 1.05 gm. N) given by mouth.
5.2		
5.1		
† 6.0†		
5.4		
9.1		4 gm. cystein given hypodermically. T 101° F. 3 to 5 p.m.
8.9		
50.3		
7.9†		
† 6.8		
5.8		Leucocytes, 20,000.
6.0		
4.5		
6.0		
6.2		
6.0		5 gm. creatin given by mouth.
5.0		
5.2		
5.2		
5.2		
		Leucocytes, 12,400.
		3 gm. sodium cholate by mouth in 4 doses.
		1.72 gm. urine-cystin given by mouth.
		Leucocytes, 17,800.

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$\frac{100 \text{ Total S.}}{\text{Total N.}}$	$\frac{100 \text{ Neutral S.}}{\text{Total N.}}$	Total C.	REMARKS.
8.0	4.6		Low protein diet. 720 grams boiled rice. 120 cc. cream.
9.0	4.5		
9.1	4.8		
17.4	5.3		3 gm. cystin given by mouth in 10 doses.
11.2	4.5		
10.8	5.6		
6.6	3.9		20 gm. glycocoll given by mouth.
9.2	4.5		
8.8	4.5		
9.2	4.7		
7.5	4.9	0.626	50 gm. casein given at 9 a.m.
7.3	4.7	2.09	
4.0	2.8	2.00	
3.7	2.6	1.04	
4.7	3.5	1.04	
6.5	4.7	1.24	
5.3	3.7		
6.7	4.1		3 gm. cystein given by mouth. Blood pressure, 136 mm.
16.5	5.4		
10.3	5.4		
9.1	4.8		

III. STUDIES OF TISSUE GROWTH.
1. AN EXPERIMENTAL STUDY OF THE RATE OF
REGENERATION IN CASSIOPEA XAMACHANA
(BIGELOW).

BY CHARLES R. STOCKARD,
Instructor in Comparative Morphology,
Pathological Laboratory, Cornell University Medical College, New York City.

29 text figures.

AN EXPERIMENTAL STUDY OF THE RATE OF REGENERATION IN CASSIOPEA XAMACHANA (BIGELOW).

BY CHARLES R. STOCKARD.

INTRODUCTION.

The suggestion has been advanced by Zeleny (1903 and 1905) that the greater the degree of injury, up to a certain limit, the more rapid will be the rate of regeneration. Zeleny's studies were based on the regeneration of the limbs in crustacea and the arms of the brittle-star, *Ophioglypha*. He was unable to offer any satisfactory explanation of why the regeneration rate increased with the amount of injury, but advanced several suggestive hypotheses which are subject to experimental test. First, he pointed out that the animal with the greater number of appendages removed might exercise the regenerating ones more vigorously than does the animal with the smaller number removed. In other words, activity should increase the rate of regeneration in animals. Child (1904) had also been led to think that some regulating influence was exerted over regenerating tissue by movement and nerve impulses in the flat-worm, *Leptoplana*. I have succeeded in devising two different ways of testing the influence of rest and activity on the regenerating tissues of the medusa and find no increase in the rate of regeneration to result from activity.

It was also suggested that the amount of available food might regulate the rate of regeneration. Those crayfish most injured have more food to draw from, since the other appendages are not present to take their share of it. Morgan (1906) has subjected this question to thorough investigation and finds that the amount or rate of differentiation of the regenerating organ is independent of the food supply, although the size of the organ is greater in well-fed individuals than in starved ones. "So long as there is enough food material in the blood or other fluids of the body to allow growth to take place at all it goes on at a rate determined by the peculiarities of each level, and largely independent of the food supply." Here Morgan mentions one of the most interesting points connected with this subject—that is, the influence of different levels of the body, or of an organ, on the rate of regeneration. In the fish's caudal fin it was found that new tissues regenerated faster the nearer the cut was to the base of the fin, and slower the nearer

the cut to the free end of the fin. At first thought this statement seems only a different way of saying, "the greater the amount of injury the more rapid will be the rate of regeneration." This is not true, however, as it was shown that the rate of regeneration varied with the shape of the cut in a manner not always correlated with the extent of the injury. Experiments will be recorded in the present paper which seem to contrast the two factors distinctly, as well as to show the peculiar influence of the level at which the cut is made.

Again, Zeleny offers the interesting conjecture that the uninjured chelæ may be assumed to exert a retarding influence upon the growth or regeneration of all the others. When one chela is removed the number of uninjured limbs remaining is greater than when both chelæ and the last two pairs of walking-legs are removed. The retarding influence with one chela gone, if the supposition be true, is greater than it is when more limbs are removed and correspondingly the rate of regeneration in the former is slower than in the latter case. Such an explanation when modified might be applied to regeneration in the salamander, the fish, and the medusa in the following way: When these animals are cut at various levels they regenerate faster the farther the cut surface, within certain limits of course, is from the extremity or limits of the animal's body. A fish's tail-fin grows faster from a straight cut near the base than from a similar cut near the end of the fin. The medusa regenerates tissue faster the farther away from the periphery the cut is made, as though the more tissue removed the less uninjured body-surface remained to exert a retarding influence.

The above considerations suggest the question of the limits of growth; as the body nears its adult or normal size the rate of growth becomes slower. It is also true that the regenerating tissue grows slower as it reaches the limits of the former body-surface. Morgan (1906) has expressed this idea as follows:

If we can find the explanation for the cessation of growth at the proper terminus we can probably find also an explanation for the difference in the rate at different levels, for, as can be shown, the two things appear to be one and the same. In other words, as the new part grows larger its materials change, and this change is of such a kind that it leads to the cessation of growth. Hence starting under different conditions at different levels the same end result will be reached in all cases, and when the terminus is reached the growth should slowly decline, as we find in fact that it does.

Emmel (1906 and 1907) has arrived at opposite conclusions after a study of regeneration and molting in the lobster from those cited above as obtained by Zeleny on the crayfish. Scott (1907), from a study of regeneration on the fins of *Fundulus*, reaches conclusions differing both from those of Zeleny and Emmel as well, since he finds that the degree of injury exerts no influence whatever over the rate of regeneration in the fish's fin. Be this as it may, the fact remains that in the salamander, the fish, the earthworm,

and the medusa the rate of regeneration does vary under various conditions of injury, but depends upon the body-level at which the cut is made.

The crustacea seem rather unsatisfactory forms for the study of such problems as the rate of regeneration. They must molt before the regenerating portion can be observed and the time between molts is often greater than the time which would be expected as necessary for the given amount of regeneration to take place. There is likely a period of cessation of regenerative growth preceding each molt. Animals which have a continuous growth of regenerating tissue seem much better adapted to these studies.

The experiments here recorded were conducted in the Laboratory of Marine Biology of the Carnegie Institution of Washington, at Dry Tortugas, Florida, during the summer of 1907. I wish to express my thanks to the Director of the Laboratory, Dr. Alfred G. Mayer, for many kindnesses extended me while there.

MATERIAL.

The rhizostomous scyphomedusa *Cassiopea xamachana* is very hardy. It attains a large size, 15 or 20 cm. in diameter, and is particularly suited to regeneration studies, as several experiments or cuts may be performed on one and the same individual where the conditions are as near similar as would be possible to obtain. Further, since all portions of the disk seem capable of regeneration one may thus work on the animal's body as well as on its tentacle-like appendages. Of exceptional importance is the fact that the circular disk will admit of variously patterned cuts which are impossible on animals with a differently shaped body. Finally, the disk pulsates rhythmically in a manner subject to the control of the experimenter, thus enabling him to test the influence of motion, or activity, on the regenerating tissue in a way not offered by any other animal yet experimented upon.

These medusæ are easily kept for long periods of time in small aquaria by merely changing the water every two or three days. They live for some time without taking food. One may collect them in abundance from the moat which surrounds the old Fort Jefferson at the Tortugas Islands. The water in this moat is about 4 to 6 feet deep, being rather stagnant at times. Here *Cassiopea* seems to thrive, and large numbers of them are to be seen lying upon the bottom with their mouth-arms turned upwards, resembling bunches of dark-colored moss.

RATE OF REGENERATION FROM THE PERIPHERY OF THE DISKS WHEN CUT AT VARIOUS DISTANCES FROM THE MARGIN.

It is well to consider first the less complex cases in which an attempt was made to determine the difference in regeneration rates from cut surfaces on the disk of *Cassiopea* at various distances from the margin. Medium-sized medusæ were selected for the experiments, and the cut consisted in each case of the removal of a peripheral strip from the entire disk. Such an

operation leaves the jelly-fish without marginal sense-organs and, therefore, its rhythmical contractions cease until a slight epithelial rim has regenerated, which serves to reestablish the pulsation. This new tissue is itself unable to contract, yet it is the seat of the stimulus which causes the disk to pulsate.

Two jelly-fish, each about 86 mm. in diameter, were cut around their entire periphery so as to remove a strip of tissue 10 mm. across (fig. 1). Two other medusæ were cut, in a similar manner as near as possible, and in addition their mouth-arms were removed, so that they were entirely de-

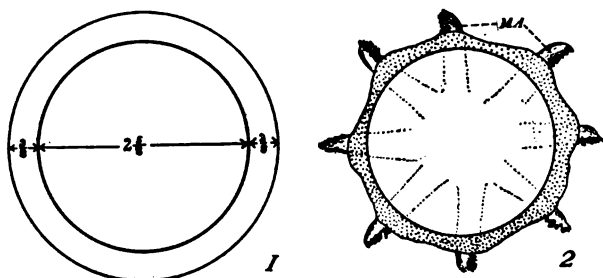


FIG. 1.—Diagram indicating method of cutting.

FIG. 2.—Stippled border shows newly regenerated tissue from cut periphery. New tissue widest where ends of mouth-arms (MA) press against it.

prived of all means of obtaining food. The former are designated in table 1 as Nos. 1 and 1A, the latter as Nos. 2 and 2A. By referring to this table the rates of regeneration from the cut peripheries may be readily ascertained for the three medusæ, Nos. 1, 1A, and 2; 2A died soon after the experiment had started. Nos. 1 and 1A were pulsating two days after the operation and in six days they had grown a rim of new tissue 3.3 mm. wide about their cut peripheries. The regenerating tissue then began to thicken and did not increase further in width until after the tenth day. On the fourteenth day the sense-organs were slightly indicated; from this time until the thirty-fifth day there was only a slight increase in the radial width of the regenerating rim until it reached about 5 mm. across, or was one-half as wide as the piece originally removed. During this period, however, the regenerating tissue was becoming thicker, until it had attained the normal thickness of the disk for the given level; further differentiation of the sense-organs was also taking place. At the same time it must be remembered that the animal as a whole was constantly becoming smaller for want of food, so that the disk of No. 1, which measured 66 mm. in diameter after the operation on June 13, measured only 40.6 mm. on July 18, or 35 days later. Thus the amount of regenerated tissue is to the diameter of the disk almost as much as the amount of tissue removed was to the original diameter after the operation was performed.

TABLE 1.—Regeneration from the cut periphery of the medusa-disk after removal of circular strips of various width.

Date.	Remarks.	Exp. 1.	Exp. 1a	Exp. 2.	Exp. 2a.	Exp. 3.	Exp. 3a.	Exp. 4.
June 13	Disk diameter before operation.	86.7	86.7	86.7	64	86.7	86.7	90
June 13	Width of removed margin.....	10	10	10*	10*	16.5	16.5	28
June 19	Width of new tissue.....	3.3	3.3	2.5	Dead.	4	4	4.2
June 21	Width of new tissue.....	3.3	3.3	2.5	5	5	4.2
June 23	Width of new tissue.....	3.3	3.3	3	6.7	6.7	Dead.
June 25	Width of new tissue.....	4.3	4.7	3.3	6.7	5.9
June 27	Width of new tissue.....	4.7	5	5	6.7	5
June 30	Width of new tissue.....	4.7	4.7	5	5	5
July 3	Width of new tissue.....	4.7	4.7	5	4.2
July 6	Disk diameter.....	51.5	45.5	29	29	39
July 6	Width of new tissue.....	5	4.5	4.7	4.6	4.2
July 9	Width of new tissue.....	5	4.5	5	4.4	3.3
July 12	Width of new tissue.....	5	5	Dead.	4.7	3.3
July 18	Disk diameter.....	40.6	33.3	(†)	31.5
July 18	Width of new tissue.....	5.1	4.7	5	3.3

Date.	Remarks.	Exp. 4a.	Exp. 5.	Exp. 5a.	Exp 5b
June 13	Disk diameter before operation.	93.3	96.6	77	
June 13	Width of removed margin.	Disk center only remained.	All of disk removed.	Small strip of disk tissue left.	Same as 5a.
June 19	Width of new tissue.....	6.7	None.....	None.....	None.
June 21	Width of new tissue.....	6.7	None.....	Regenerating from disk tissue.	Regenerating from disk tissue.
June 23	Width of new tissue.....	6.7	None.....	Same as on 21st.	Same.
June 25	Width of new tissue.....	5.3	None.....	Film of tissue over entire top.	Almost same as 5a.
June 27	Width of new tissue.....	(thick) 4	None.....	Same as on 25th.	Same.
June 30	Width of new tissue.....	3	None.....	As on 25th.	As on 25th.
July 3	Width of new tissue.....	3	None.....	As on 25th.	Dead.
July 6	Disk diameter.....	21
July 6	Width of new tissue.....	4	None.....	Thin film over top (aboral) surface of mouth-arms, regenerated from small pieces of disk tissue.
July 9	Width of new tissue.....	3.3	None.....	
July 12	Width of new tissue.....	4.6	Dead.....	
July 18	Disk diameter.....	20
July 18	Width of new tissue.....	6.5

* Mouth-arms also removed.

† Arched aborally, not measured.

It was observed that those portions of the regenerating rim which were touched or pressed against by the mouth-arms of the medusæ regenerated faster or grew out wider in a radial direction than the intermediate portions which were not so pressed by the arms (fig. 2). This condition may possibly be due to the mechanical pressure of the mouth-arms against such places causing them to thin or flatten out, thus giving a more rapid radial growth, whereas the entire mass of tissue may be no greater here than from other parts of the regenerating surface. I made no observations, however, at the time of the experiments to ascertain whether the new tissue was thin-

ner at these places where the mouth-arms pressed. Regeneration also proceeded at a faster rate in the irregularities of the cut surface. This case will be fully considered in a following section.

No. 2, which was cut in the same way as Nos. 1 and 1A and in addition had all of its mouth-arms removed, regenerated somewhat more slowly at first, although obviously the most injured of the three. Later, however, it showed almost as much regenerated tissue as either of the other two. During the observations this medusa showed a very peculiar condition; the periphery of the disk became arched aborally and the regenerating tissue was thus also directed aborally, being finally so folded over that the animal became cup-shaped (fig. 3). The regenerating tissue then grew toward the center, and by fusing the edges of its periphery changed the cup into a hollow sphere. This condition was also observed in several other experiments and may be explained thus: The muscles being slightly out of the nor-

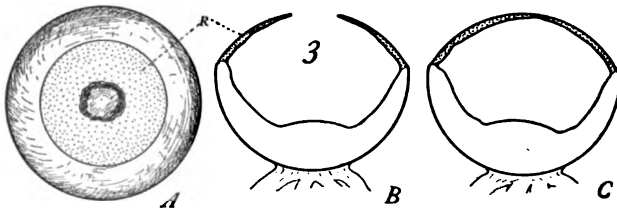


FIG. 3.—*A*, top view of aborally arched disk; new tissue (*R*) regenerating from cut periphery grows toward center. *B*, cross-section of such specimen. *C*, new tissue completely fused over top, converting former disk into hollow sphere.

mal condition of coördination, those expanding the disk act more strongly than the oral contractors and the periphery is thus gradually directed more and more aborally. The new regenerating tissue has a tendency to fuse if two of its surfaces are brought together so that when its periphery is folded aborally and the edges come together they fuse and form the hollow sphere. A similar balloon-like condition has been recorded by Hargitt (1899) in *Gonionemus*. Hargitt was unable to produce such a condition artificially, although he tried in several ways to do so.

Two other medusæ, designated in the table as Nos. 3 and 3A, had a strip 16.5 mm. wide taken from the peripheries of their disks. These disks are, therefore, more injured than the first and they are also cut at a deeper level. After 6 days they had regenerated a rim of tissue slightly wider than that of Nos. 1 and 1A, and after 10 days the rims of the latter were only half as wide as those of Nos. 3 and 3A. From this time the periphery of No. 3 became abnormally arched and its regeneration was slightly modified, yet it continued ahead of Nos. 1 and 1A. The disk of 3A remained flat and the regenerated border here increased rapidly in width for 12 days and then commenced to thicken and ceased to grow in width; the sense-organs began

to appear after 23 days. The disk had resumed its rythmical pulsation in 2 days after the operation. It will be found by a study of the table that after about 12 days the regenerated tissue began to decrease in width. This fact may be explained by the thickening which the new tissue commences to undergo at this time, or again it may result from the causes which tend to make the entire disk gradually decrease in diameter, until after 35 days it is little more than half as large as it was when the experiment began.

Nos. 4 and 4A were cut so that only the center of the disk covering the bases of the mouth-arms remained. From No. 4 a strip almost one-third as wide as the entire diameter of the medusa was cut away. This disk was 90 mm. in diameter before the operation and only 34 mm. after the removal of the strip. It must also be kept in mind that the cut surface at this level is very thick, since the disk is thickest at the center and becomes thinner as the margin is approached. No. 4 died soon after the experiment started, as is indicated in the table. No. 4A was healthy and within 6 days had regenerated a rim of tissue from its cut surface which was almost twice as wide as that observed in any of the above experiments. After 12 days, here again, the regenerated strip ceased to increase in width, but continued to become thicker. Finally, as is shown in table I, the rim of new tissue actually began to decrease in width as it had in 3A.

The deep-cut surfaces when regenerating first grow a wide, thin rim of tissue which finally begins to thicken at the expense of radial growth till the normal thickness of the disk at the given level is reëstablished. It will be seen that regenerating tissue from a cut surface near the disk margin widens slowly, but almost continuously, as at this level the disk substance is very thin and no subsequent thickening of the regenerated tissue is necessary.

Three medusæ were now cut so that in two individuals only a small bit of disk remained attached to the mouth-arms and in the third the entire disk was removed. The object of such operations was to ascertain whether the mouth-arms were able to regenerate a disk, or disk-tissue. It was found that the very small portions of the disks which remained would regenerate new tissue, but the mouth-arms were incapable of regenerating from their bases, although they healed the wounded surfaces and lived for 29 days after the entire disk had been removed.

Other experiments on removing strips of various widths from the periphery were made and results closely similar to those above were obtained. One must then conclude that the disk of *Cassiopea* begins to regenerate its margin at a faster rate the nearer the cut is to the center of the disk. A small individual regenerates proportionately faster than a large one. These results are closely similar to those obtained by Morgan on the salamander, fish, and earthworm, and by comparison show that the rates of regeneration differ at different levels of the body, and further that (as in embryonic growth) the nearer the normal body-size or form is approached the slower

will be the rate of the regenerating growth. Miss King (1898) finds in *Asterias* that the rate of regeneration is greatest from the disk and decreases directly towards the tip of an arm. It is also true that those medusa-disks cut nearer the center are the greatest injured and according to Zeleny would be expected to regenerate their removed tissue fastest, just as they really do. It so happens that the difference in level and the amount of injury are often closely associated. I shall, however, cite an experiment below which serves to contrast the two and shows the level of the cut to be the more important factor in regulating the rate of regeneration.

RATE OF REGENERATION FROM DIFFERENT PARTS OF VARIOUSLY SHAPED CUT SURFACES.

For the study of problems relating to the rates of growth from surfaces partially cut as compared with those entirely cut, and the rates of growth from different parts of the same cut surface, *Cassiopea* offers exceptional opportunity, since the disk-body itself may be cut in sundry patterns and the regeneration rate observed in the several cases. Morgan (1902 and 1906), from a study of regeneration in the fish's fin, has contributed a number of valuable observations bearing on the question in point. The caudal fins of *Fundulus* and *Carassius* were trimmed in different ways, and it was found that partially cut surfaces regenerated slower than entire surfaces cut at the same level; also that new tissue grew out at a faster rate from certain parts of all cut surfaces than from other parts. Since Morgan's experiments were confined to the manner of regeneration from fins or appendages, I determined to make similar cuts upon the disk or "body" of the medusæ to ascertain whether the same principles in regeneration would hold. The results show not only that the same manner of regeneration is adhered to in the body and in the appendages of the two animals, but further, that the forces controlling or determining the regeneration rate on various parts of the cut surfaces act similarly in animals as different as fish and medusæ, almost at the opposite ends of the animal series.

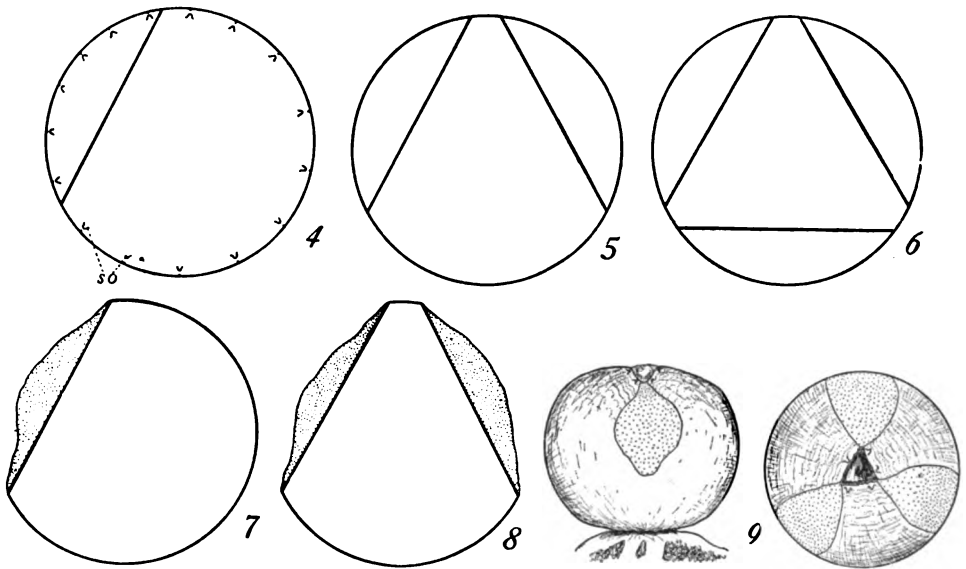
Straight cuts were made upon the disks of medusæ in the following ways: First, a single piece was cut from the disk, as shown in figure 4. Second, two such pieces were cut off as indicated in figure 5, and lastly three pieces were removed as in figure 6. Five individuals were cut in each way and different-sized pieces were removed. The course of regeneration followed by each of the cuts in all of the 15 medusæ was practically the same. The history of one set, consisting of one of each kind of individual, will answer for all.

The specimen having one cut will be designated as A, the two cuts as B, and the three cuts as C. From A a portion of the disk was removed that measured 32 mm. wide at its broadest place and included 6 of the 16 marginal sense-organs. Four days later the regenerated tissue from the cut

was shaped as indicated in figure 7. The middle part of the cut, which is the deepest part and nearest the disk center, regenerates faster than the sides. After a number of days the middle part goes a little slower and when the cut is 20 days old the regenerated tissues from different parts of the cut surface are about the same widths, although the middle portion is the thicker.

Sense-organs commence to form from the new tissue at this time. The new tissue, being weaker than the other parts of the disk, is sometimes pulled aborally and somewhat folded or puckered, so that it is difficult to measure accurately, though during the first 25 days of the experiment the regeneration rate at different portions of the cut may be accurately measured.

The manner of regeneration from the two cut surfaces of B is identical with that from the single cut of A. In both, then, the rate of regeneration



FIGS. 4, 5, 6—Diagrams indicating ways in which disks were cut to give one, two, and three straight cut surfaces. SO, sense-organs.

FIGS. 7, 8.—New tissue (stippled).

FIG. 9.—Top and side views of disk cut as shown in fig. 6. During regeneration the intact corners became aborally arched, modifying the manner of growth and producing hollow spheres with opening at top.

is retarded at the marginal corners of the cut, so that the mid-portion grows ahead of the lateral parts (see fig. 8).

The three cut surfaces of C (fig. 6) follow the same course of regeneration as do those of A and B. Disks cut in this way, however, seem especially inclined to turn their three intact corners aborally, and in so doing the cut surfaces, instead of remaining straight form angles. It will be shown more in detail later that regeneration proceeds much more rapidly in an angular cut than from a straight surface, since the two sides of the

angle seem to reinforce one another in regeneration, a kind of summation of regeneration occurring. Through such a process the disk is converted into a ball-shaped body with a small triangular opening at the top, where the three uninjured corners are brought almost together (fig. 9).

It will be recalled that the fins of the gold-fish and *Fundulus*, when cut straight across, begin to regenerate their new tissues faster in the middle of the cut and slower near the corners, a fashion identical with that followed by the disk of the jelly-fish.

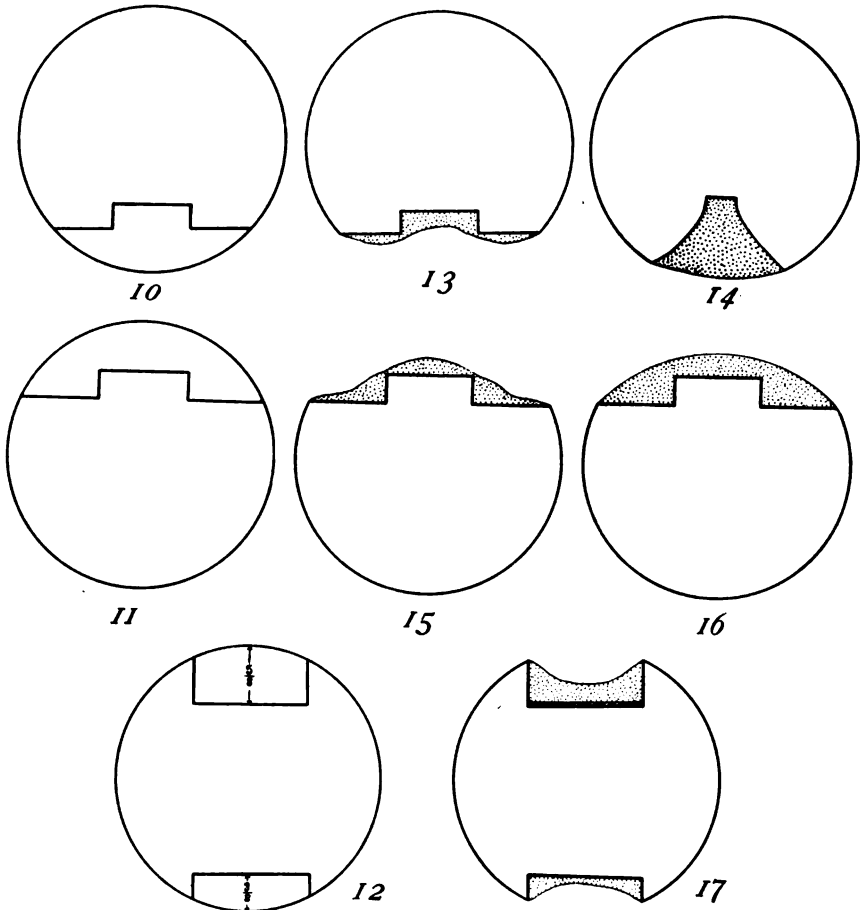
The medusæ-disks were next cut in such patterns as to give what Morgan has termed "partial cut surfaces" (figs. 10, 11, and 12). Such cuts were varied in the width of their different parts as well as in depth. Many individuals were prepared in the several ways.

The deep part of the cut shown in figure 10 must be wide, since it shows a strong tendency to close its walls together after a week or two (fig. 14). The history of the regeneration from such a cut surface may be recorded in detail. The cut was made so that the bottom of the deep part was 23 mm. from the peripheral margin at its most distant point; this part was 26 mm. in width; the lateral shallow parts of the cut were each 19 mm. wide and 10 mm. below the margin at their middle point. Four days after the operation the regeneration was perceptibly greater from the deep-cut surface than from the lateral shallow surfaces, and within six days the middle part had almost overtaken the lateral surfaces (fig. 13). The regeneration in the deep cut really takes place from three surfaces, the bottom and the two sides of the cut, as here there is free opportunity for lateral regeneration, thus differing from the case of the fish's fin, where the fin-rays seem to prevent lateral regeneration, since they are only capable of growing out from the stumps of the old rays.

Ten days after the operation there was 13 mm. of regenerated tissue from the deep cut and only 10 mm. from the lateral shallow parts. After 14 days the deep cut had become so pulled together that there was only 5 mm. between its original walls. When 20 days old the regenerated tissue had rounded across its free margin and was now growing out as one piece. After 23 days the old sides of the deep cut were only 3.3 mm. apart; the regenerated tissue over it measured only 10 mm. and over the shallow parts 7 mm. This loss in width may be either due to the thickening of the new tissue which is taking place, or may be on account of the general decrease in size which the medusa has undergone, measuring now 63 mm. in diameter, whereas it was 77 mm. across when the experiment began. The new tissue from the deep cut after the twenty-third day began again to increase slowly in width until when 35 days old it was 14.5 mm. wide and that from the shallow parts was 8 mm. The original walls of the deep cut were almost drawn together, being only 2.3 mm. apart. The entire cut had tended to contract, so as to take an angular form, as illustrated in figure 14. All

medusæ operated on in this fashion regenerated similarly to this one. Their manner of regeneration may then be briefly summarized as follows:

The rate of proliferation of new tissue is faster from the deep partial-cut surface and slower from the lateral surfaces. The angles of the deep partial cut assist in the regeneration process and thereby help to make it proceed faster from this portion of the cut, whereas the corners of the lateral cuts seem to exert a retarding influence over the rate of regeneration



FIGS. 10, 11, 12.—Diagrams showing manner of cutting medusæ disk to test regeneration rates from partial cut surfaces.

FIGS. 13, 14.—Stippled areas indicate course of regeneration from cut surface of pattern, fig. 10.

FIGS. 15, 16.—Course of regeneration from such a cut as shown in fig. 11.

FIG. 17.—Showing manner of regeneration from fig. 12.

(see fig. 13). Such a conclusion is identical with that reached by Morgan in his study of the regeneration from similar cut surfaces on the fish's fin.

We may now consider regeneration from surfaces cut in practically the opposite manner from those just recorded. The lateral cuts are deep, with a high middle tongue-piece (fig. 11). Many medusæ cut in this fashion regenerated tissue in a similar way. The exact history of one of the individuals is as follows: The disk was cut so that the lateral surfaces were 26 and 41 mm. wide, respectively, and the high tongue-piece between them was 14.5 mm. wide and 10 mm. high, or above the level of the side cuts (fig. 11). Six days after the operation the newly proliferated tissue was widest on the two side portions and narrow from the middle piece. The corners of the high middle part seemed to exert a retarding influence on the regenerative processes, as did also the outer or marginal corner of the lateral cuts. The inner corners of the side cuts were, on the other hand, the places of greatest regeneration, as no doubt the lateral and basal surfaces both contributed to the process (fig. 15). Nine days after the operation the regenerated tissue from the lateral cuts was 5 mm. wide, while that from the middle piece was only half as much. On the twelfth day the conditions were about the same. The fifteenth day gave the side parts 7 mm. of new tissue, while the middle part had proliferated tissue only 2.3 mm. wide. At this time the old border of the middle piece is 8 mm. wide, while the lateral parts are 16 and 5 mm. respectively. When 21 days old the regenerated tissue had rounded its border (fig. 16) and measured 7 mm. deep over the side cuts and 3.5 mm. over the middle part. From this time until the twenty-seventh day the middle part continued to grow out new tissue, while the side portions seemed to have completed themselves.

Regeneration from such a cut surface may be thus summarized. The lateral cut surfaces produce new tissue faster than the high middle piece. The outer corners of all the cut surfaces seem to exert a retarding influence on the rate of regeneration, while from the inner corners of the lateral cuts new tissue is formed at a very rapid rate, which is probably due to a summation of regeneration. It will be again recalled that an identical condition exists in the regeneration from similar cuts on the fish's tail.

Medusæ were also cut in such a way as to test the rate of growth at different levels on one and the same individual. Here, obviously, the conditions of nutrition and vigor must be as nearly identical as possible. At one place on the rim of the disk a piece was cut out which was 10 mm. deep at its broadest part. Opposite this cut, or 180° away, a second piece of the disk, including an arc of the same extent, was cut away to a depth of 16 mm. from the highest point of the arc (fig. 12). When one cut is narrower peripherally than the other, the rates of regeneration are not readily compared, since regeneration proceeds more rapidly from a narrow cut than from a wide one at the same level.

After six days the regenerating tissue was broader from the deep than from the shallow cut, although here it has a thicker base of tissue to grow

from; this is also true for the fish's fin, where the deeper cut has a thicker base. Two measurements of the regenerating tissue were made, since the thick base was not exactly a perpendicular surface. The one was from the edge of the old tissue on the aboral surface to the edge of the new tissue, which measured 5 mm. over the deep-cut surface; the other measurement was from the oral border of the old tissue to the margin of the regenerated tissue, 3.5 mm. wide. The rate of regeneration was fastest at the corners in these cuts, being 7 mm. wide at this place in the deep cut. The shallow cut showed 1.5 mm. of new tissue from its middle and 5 mm. from its corners (fig. 17). After 9 days the deep cut had regenerated tissue 7 mm. wide from its middle, while the shallow cut showed only 3.5 mm. of tissue. Both of the cuts were at this time 13 mm. in width peripherally. When 18 days old the cuts were 10 mm. across between the vertical edges of the old tissue, the deep cut had regenerated new tissue 7 mm. wide and the shallow 3.5 mm., or half as much. Here again regeneration proceeds in one and the same individual at a faster rate from the cut surface at the level nearer the disk-center than from a similar more distal cut.

I may now cite an experiment which was made to test whether medusæ would regenerate their sense-organs faster when consecutive ones were removed or when alternate ones were cut away. The experiment threw no light on this question, but the result was curious and for this reason may be mentioned. Two healthy medusæ, one with 16 and the other with 17 marginal sense-organs, were treated as follows: Four adjacent sense-organs were removed from one part of the disk and three alternate ones from the region opposite these. After 23 days no definite trace of regenerating sense-organs could be detected, so all of the remaining old sense-organs were cut away to ascertain whether the new ones were sufficiently regenerated to maintain the pulsation of the disks. The disks became perfectly still after the last one of the original sense-organs was cut off, and only after a period of 6 days was one individual slowly pulsating. This is peculiar, as when the entire peripheral border with all sense-organs is removed the newly regenerated tissue causes the disk to pulsate usually after two or three days. Further, a number of medusæ with regenerated margins had produced sense-organs from their new tissue, while the two above had not regenerated them from their old bases.

REGENERATION AFTER THE REMOVAL OF PIECES OF ORAL EPITHELIUM OF DIFFERENT SIZES AND AT DIFFERENT DISTANCES FROM THE DISK-CENTER—THE QUESTION OF "REGENERATIVE PRESSURE."

These experiments were carried out with the hope of testing whether or not "regenerative pressure," in the sense Morgan (1906) used the term, actually exists and exerts itself from the center radially to the periphery. In other words, is this force felt more towards the center and gradually

less, as the limits or periphery of the body is reached? This pressure is responsible for the "gradual slowing of regeneration as the normal form is approached, and it is apparent that this retardation will be the same, whether it occurs near the end of an old part or as a new part approaches completion." If this be true it ought also to follow that the pressure conditions of regenerative forces are greater in the center than at the periphery of the disk in *Cassiopea*.

In the experiments, only the oral epithelium and thin superficial muscle-layers were removed. It may be that such experiments are not conclusive, since this pressure might exert itself outward from the face or cross-cut area only, and not so clearly on the surface. At any rate, as will be seen, the results do not lend particular strength to the idea of greater regenerative pressure near the center.

Six medusæ were operated upon as follows: From the oral surfaces of two individuals, Nos. 1 and 1A, two rectangular pieces of epithelium and underlying muscle were removed. The removed tissues had the same width in a radial direction and were equidistant from the periphery, while one piece was longer than the other in the direction parallel to the circumference of the disk (fig. 18). If the pressure exerts itself only in a radial direction, then the two cuts should regenerate at the same rate independent of their peripheral lengths, since they are equally wide. Two other medusæ, Nos. 2 and 2A, had two equal-sized pieces removed from their oral surfaces, one piece being nearer the center than the other (fig. 19). The last two, Nos. 3 and 3A, had one piece running in a radial direction cut from each, as seen in figure 20.

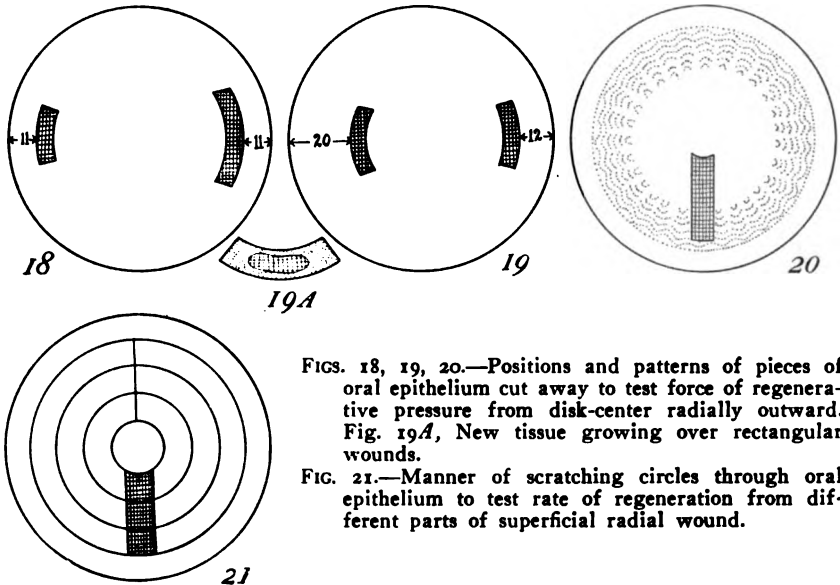
Mayer (1906) has shown that when the epithelium is scraped away on the oral surface of *Cassiopea* an electrical stimulus applied on one side of the abrasion is unable to pass over and stimulate the tissue on the other side. As soon, however, as a very delicate layer of tissue is regenerated over the cut place the stimulus will be transmitted across. This affords a delicate means of detecting the first trace of regeneration.

Twenty-four hours after the above operations No. 3 was scratched on its oral surface so as to divide it into a series of concentric rings (fig. 21). The rings were then scratched across at a place opposite the removed radial strip. The rings of tissue were thus broken at one place, so that no impulse could pass from one of their halves to the other unless tissue had regenerated over the radial injury sufficiently to conduct the stimulus. It would be expected that the inner ring should be the first to conduct. None of the injuries had regenerated sufficiently after 24 hours.

Two days after the operation Nos. 1 and 1A did not transmit across their injuries. No. 2 transmitted the stimulus across the inner area only, although this was equal in extent to the more peripheral injury. The clear, transparent regenerating epithelium could now be seen, and it was noticed

that the growth was greatest at the two ends instead of in the radial direction (fig. 19A). This is probably due to the corners being nearer together at these ends, and regeneration takes place from both sides of the angles, such a summation causing it to proceed faster.

Four days after the operation all of the scars had regenerated tissue sufficiently to cover them completely over. The smaller places had regenerated sooner than the larger ones, yet a comparison of rates of regeneration is difficult to make, since the wounds tend to draw their walls together and



FIGS. 18, 19, 20.—Positions and patterns of pieces of oral epithelium cut away to test force of regenerative pressure from disk-center radially outward. Fig. 19A, New tissue growing over rectangular wounds.

FIG. 21.—Manner of scratching circles through oral epithelium to test rate of regeneration from different parts of superficial radial wound.

thus close at the same time that the regeneration is in progress. On the whole this experiment is unsatisfactory.

A somewhat similar experiment was arranged to test the rates of regeneration of epithelial coverings over wounds of different sizes and others of the same size at different distances from the disk center. The sizes of the holes were regulated by means of a sharp cork-borer, which could be used to cut out small circles of exact diameters. Nos. 1 and 1A each had three circular wounds 10 mm. in diameter at 10, 16, and 20 mm. from the margin. Nos. 2 and 2A had three circles scraped, each about 24 mm. from the disk margin and over radii leading to the sense-organs. The circles were 7, 8.5, and 10 mm. in diameter. On 3 and 3A four circles each, 8.5 mm. in diameter, were scraped, 20 mm. from the margin, two of the wounds being over radii leading to sense-organs and two midway between such radii. All four are, however, immediately below radiating canals, so that the difference in regeneration rate, should any be observed, might be attributable to their different nervous connections (fig. 23).

Two days after the operation Nos. 1 and 1A showed their outermost circles with regenerated films about half over them; the inner circles were in the same condition, but the two circles occupying intermediate distances from the margins had regenerated films of epithelium which entirely covered the wounds. In No. 2, where the three circles were equidistant from the margin but of different diameters, the largest and smallest circles were still not completely covered over, while the one of intermediate size was entirely covered. In 2A all of the circles had regenerated coverings entirely over them. Nos. 3 and 3A showed all of the holes to be 5 mm. in diameter; thus they had become contracted to little more than half of their original size. Those on the sense-organ radii seem a little further covered than those on intermediate radii, though there is very little difference at all.

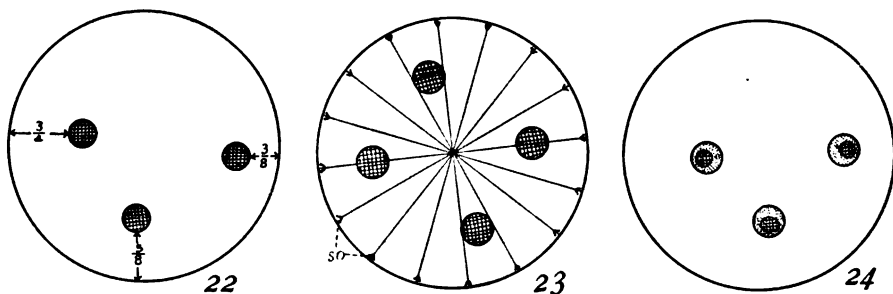


FIG. 22.—Medusa disk with 3 equal sized circular wounds at different distances from margin.

FIG. 23.—Disk with 2 circular wounds over radii leading to sense-organs, and 2 exactly similar wounds between sense-organ radii. SO, sense-organs.

FIG. 24.—Regeneration from 3 circular oral wounds.

With these circular cuts one eliminates the angular regeneration factor mentioned in the experiments above, and it was noted in all cases that the film was widest from that area of the circumference toward the disk center (fig. 24). This condition would be expected on the hypothesis of greater regenerative pressure near the disk center, though the deeper level of the cut at this part is a better explanation.

Three days after the operation all of the circles were entirely covered over. These experiments are also difficult to draw conclusions from, since the wounds have a tendency to contract while they are healing and regenerating new tissue. Those nearest the disk center contract most. Thus one might believe them to be more rapidly producing the new tissue.

THE RATE OF REGENERATION FROM DIFFERENT AREAS ON TAPERING PERIPHERAL STRIPS AND REMAINING PART OF DISK—CONTRASTING THE LEVEL OF THE CUT AND EXTENT OF INJURY.

The circular medusa disk offers exceptional material for certain operations that could not be carried out on animals having a differently shaped body. It has been found difficult to perform an experiment which would clearly contrast the rate of regeneration from certain levels with the rate from parts more or less injured. According to Zeleny the rate of regeneration will be faster the greater the injury up to a reasonable limit, and according to Morgan's pressure and growth idea the rate varies at different levels, being slower as the level is nearer the normal body limits. The conditions are usually open to either interpretation, since the least injured animals are the ones with less body tissue removed and necessarily nearer the normal body limits than those with more tissue removed.

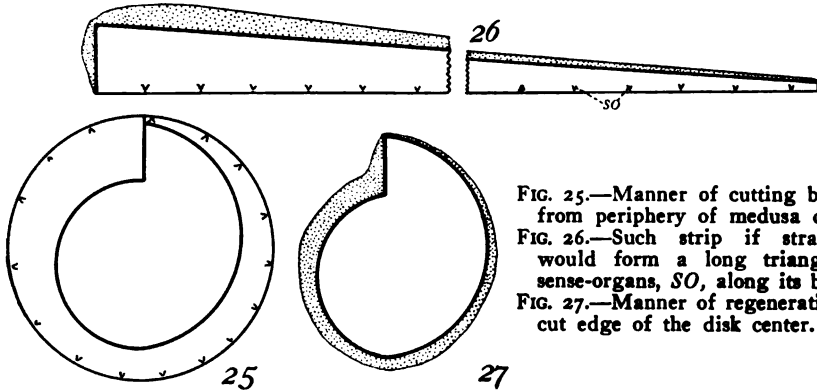


FIG. 25.—Manner of cutting bias strip from periphery of medusa disk.

FIG. 26.—Such strip if straightened would form a long triangle with sense-organs, *SO*, along its base.

FIG. 27.—Manner of regeneration from cut edge of the disk center.

If a medusa disk is so cut that a strip wide at one end and narrow at the other is removed from the entire periphery, then regeneration will take place from the entire cut surface of the strip and also from the cut margin of the remaining disk center (fig. 25). *The strip* is most injured, one may say, at its narrow end, as obviously here more of the disk has been removed from it, and it is least injured at its broad end, as here less tissue has been cut away. The wide end of the strip, however, has more raw tissue exposed by the cut, as the disk is thicker near the center and gradually thinner toward the periphery. One might claim that those portions with the most raw tissue exposed were the most injured; therefore the strip and disk center were equally injured along corresponding regions of the cut. It seems more logical, however, to consider the object most injured from which a greater amount of its original body tissue has been separated. The remaining *disk center* is most injured where it was deepest cut or on that part from which the wide end of the strip came, and it is least injured where the narrower part of the strip came from.

The rate of regeneration from the strip, which when straightened forms a long triangle, is fastest at the wide end and is gradually slower as the narrow end is reached (fig. 26). In other words, *it is fastest from the part of least injury*. The regeneration rate from the disk part is most rapid in the deep cut and slower as the cut approaches the margin (fig. 27). Here, then, it is *fastest at the place of greatest injury*. In both cases, however, regeneration is fastest at the deepest, or same, level, and slower as the level nears the margin. It is of interest to note that the *regeneration in both directions, toward the periphery and toward the center, proceeds at almost the same rate from the same level*.

It might be claimed that the narrow end of the strip did not have sufficient material for more rapid regeneration, but this is scarcely possible, since the entire strip is in a healthy, vigorous condition and the narrow end might easily draw on other portions for food material. The rate of regeneration at the narrow end is due to its level, and is usually the same as that from the corresponding place on the center disk, or even in some cases the rate of regeneration from the narrow end may exceed that from the same cut area of the disk.

After removal from the disk the strip continues to pulsate, thus having a twisting serpentine motion which often causes it to twist or become folded. Bends and folds form angular-like places along the cut surface and, as mentioned in previous sections of this paper, the shape of the cut exerts an influence on the rate of regeneration. This source of error has been kept in mind and the regenerating tissue from the strips carefully measured on all parts. It was evident that regeneration proceeded in exactly the manner cited above and was oftentimes twice as much from the wide as from the narrow end of the strip within 5 days after the operation.

This experiment seems to contrast in a way the influence due to the degree of injury and those exerted by the different levels of the animal's body upon the rate of regenerative growth. If this be true the level at which the cut is made is the more important factor of the two, and if the extent of injury exerts any influence upon the rate of regeneration it is a secondary influence and probably due only to the fact that the amount of injury and level are closely associated. The greater the injury to a medusa the closer the level is to the disk center.

That such an experiment on the medusa-disk is to be freely compared with experiments in which different numbers of appendages are removed will probably not be generally admitted. I should not like to be understood as claiming that the narrow end of the "strip" bears a similar relation to the wide end as that which the animal with many appendages removed does to the one with few.

This experiment serves further to indicate that activity and rest are negative factors in determining the rate of regeneration. The bias-cut strip is in periodic pulsation from the time it is removed from the central

portion of the disk, while the latter does not pulsate until a sufficient rim of new tissue has formed around its cut edge, usually requiring about two days after the operation. Yet the rate of regeneration is practically the same from corresponding parts of the two pieces. The physical condition of the strip and center piece must be closely similar, since they are parts of the same individual. It is important to remember here that the sense-organs and a part of the nerve-ring accompany the strip, while the central part has much less nervous tissue; yet this fact seems to cause no difference in the rate of regeneration of new tissue from the two pieces. The question of the influence of activity and rest will be attacked in a more conclusive manner in the next section.

REGENERATION DURING ACTIVITY AND REST—WITH AND WITHOUT RHYTHMICAL CONTRACTIONS.

Zeleny has suggested, with reservation, that when several appendages are removed, the effort to use the regenerating ones, or exercise, may cause the buds to grow faster. The general question of the influence of the nervous system on regeneration is an important one. Since *Cassiopea* is an animal with a rhythmically pulsating movement, it seemed likely that an experiment might be so arranged as to test the influences of activity and rest on the rate of regeneration. With such an idea in view a number of experiments were performed, the most satisfactory being recorded below.

A ring about 20 mm. in width was cut from the margin of the disk. The sense-organs were removed from half the periphery of this ring, while equal-sized pieces of tissue between the sense-organs were cut from the other half, the degree of injury thus being practically equal on the two halves. If, then, the epithelium was scraped across between the sense-organ half and the other without sense-organs, the first half continued to pulsate, while the latter comes to rest (see fig. 28, A). Such a ring will regenerate tissue toward the center until the circular space is covered over. Careful measurements were made on six preparations of this kind with two controls which had the entire ring in motion and two others with all the ring at rest. Comparing all measurements, it seems as though the tissue was regenerated at approximately equal rates from the two halves (fig. 28, B). From this experiment one is unable to find any reason to believe that activity or effort is capable of accelerating the regeneration rate. In this animal the rate of regeneration seems to be independent of the nervous impulse necessary for activity. The experiments given in the previous section, as well as those to be considered in connection with the influences of various salt solutions on the rate of regeneration, also furnish some evidence on the question of activity and rest as determining factors.

Child (1904) has, in a number of contributions upon the subject of Regulation, held that activity exerts a marked influence upon the manner of regeneration. He has also claimed that the nervous system exerts an in-

direct influence over the regeneration rate through its control of the animal's movements. The experiments recorded above, however, seem to contrast (in a manner only possible on some such unique form) the effects

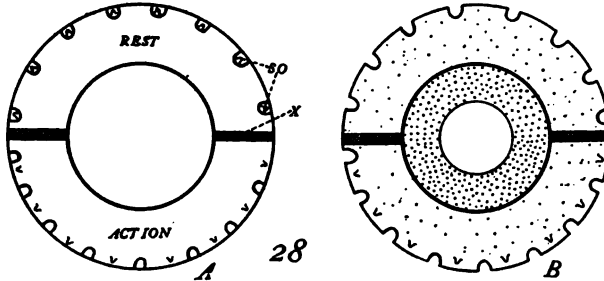


FIG. 28.—Ring from which disk center has been cut away. *A*: *so*, sense-organs cut away from upper half; from lower half equal-sized pieces have been removed. *X*, insulation between halves. *B*: Stippled area shows rate of regeneration to be equal from the two.

of activity and rest, and the results show in a decided way the negative influence of the two conditions. If such facts apply to the animal kingdom generally, then Child's idea that the regulation of growth is controlled by these factors is insufficient to account for the observed phenomena.

THE RELATIONSHIP BETWEEN THE NUMBER OF REMOVED MOUTH-ARMS AND THEIR RATES OF REGENERATION.—DOES THE REGENERATION RATE OF AN APPENDAGE VARY DIRECTLY WITH THE EXTENT OF INJURY?

The conclusion that the animal with the greater number of removed parts regenerates each part more rapidly than does the one with the lesser number of removed parts was suggested by Zeleny (1903, 1905) and supported by his studies on several forms. He (1903) found in *Ophioglypha*, the brittle-star, that when three or four of the arms were removed from an individual each arm would regenerate faster than would the arms of an individual with a smaller number removed. The difference in rate was sufficient to be shown in his text-figures (1905, fig. 4). The arms of this star-fish are all similar in size and form, and thus the regenerating bud from the base of any one may be compared with that from the base of any other. Such an animal also regenerates the arm-buds by a process of continuous growth. No criticism that I know of can be cited against these observations on *Ophioglypha* nor against Zeleny's conclusion that in this species at least the rate of regeneration is fastest in the series which has undergone the greatest injury, except possibly the objection that the series consisted of too few individuals. It may be that while in one group of animals results similar to Zeleny's would obtain, in another different results might follow. This can only be ascertained by further observations.

It will be shown below, from a study of several series of *Cassiopea*,

in which all of the mouth-arms are, likewise, almost similar in size and form and regenerate by a process of continuous growth, that wide variations are presented in the rate of regeneration of the several arms. The series with a few removed arms may regenerate each arm as rapidly as do those with a greater number of removed arms. An individual with several arms cut away often shows as great variation in the regenerating rates of the different arms as is found on comparing the average rates of individuals with few and many arms removed. Here it may be emphasized that the variation in regeneration rates of the several arms of one and the same individual (a variation shown to exist only slightly in most of Zeleny's tables, but which is very striking in some of mine) is an index to the dangers which arise when one compares the average rates of regeneration found in several different individuals. Miss King (1898) also states in her study of regeneration in *Asterias* that "the rate of growth of the new arms is ordinarily unequal when a disk regenerates two or more at the same time."

Zeleny has studied three forms of decapod Crustacea—*Gelasimus*, *Alpheus*, and *Cambarus*, the crayfish. He realized that so few individuals of *Gelasimus* and *Alpheus* were available for final comparison that it was unsafe to draw general conclusions. In these two forms the chelæ are of unequal size and in *Alpheus* they undergo a reversal upon removal of the larger one. (See Prizibram, 1901, and Wilson, 1903.) Zeleny cites such facts as introducing complications in this study. The crayfish, *Cambarus*, has the two chelæ of equal size and for other reasons also is better suited to regeneration experiments. After a careful study of 77 individuals, 61 of which were available for the final comparison, Zeleny concludes:

That in the series with the greater degree of injury each chela regenerates more rapidly than the single removed chela of the series with the lesser degree of injury. Likewise [and this is the point that I shall attempt to show the importance of in interpreting Zeleny's experiments] the members of the series with the greater injury molt more rapidly than those of the series with the lesser injury.

This work on the Crustacea seems to me to be open to criticism. Such animals must molt before the regenerating bud is observed. The time elapsing between the operation and the first molt varied from 27 to 181 days, and according to Zeleny, "the amount of regeneration of the right chela at the end of the first molt is the same, no matter what the degree of injury may be." Further, "The specific amount of regeneration at the end of the first molt after the operation is a constant which is not affected by the time of the molt, the size of the animal, or the degree of other injuries to the individual." It seems almost inconceivable that one animal should thus require nearly seven times as long to regenerate a bud of a given length as was necessary for another to grow a similar bud.

Zeleny now takes this "constant" (specific amount of regeneration at the end of the first molt) and divides it by the number of days between

the time of operation and the first molt and considers the quotient as the "specific rate of regeneration per unit of thoracic length per day." As quoted above, "the members of the series with the greater injury molt more rapidly than those of the series with the lesser injury." Thus it clearly follows that any "constant" divided by fewer days (a smaller number) will give a greater quotient (specific rate of regeneration per day) than the same "constant" divided by a greater number of days. The calculations included in Zeleny's tables 6 and 7 (1905) then, to my mind, fail to show that the greater degree of injury causes a faster regeneration, but merely that those most injured molt sooner after the operation; and since all regenerate the same specific amount at the end of the first molt regardless of the time, of course the ones molting soonest will appear to grow that specific amount quickest.

A more plausible line of reasoning would seem to be the following: Since all have the same specific amount of regeneration after the first molt, whether it takes 27 or 181 days for the molt to occur, this specific amount probably begins to be formed soon after the operation and continues until it is prevented by the chitinous covering of the crayfish, just as the animal's increase in body-size is always checked by the amount of expansion possible within its inflexible case. When the growth is so checked it must stop and remain quiescent until the molt occurs. The full amount of growth may be attained in 10, 20, or 30 days—no one can say—but after it is once attained all succeeding days until the molt occurs are not days of growth at all, but merely a quiescent period. That some such process as this is followed is strongly suggested by the fact that the specific amount of regeneration is a "constant" for all at the time of the first molt.

It is not at all certain, then, that the regeneration is continuous throughout the period elapsing between molts; therefore, one is in error to divide the specific amount of regeneration, a "constant," by the number of days between molts and to consider the quotient obtained as the specific rate of regeneration per day. The columns of specific rates in Zeleny's tables 6 and 7 mean nothing, unless it can be proven that the regeneration is continuous during all of the 27 or 181 days, and since the columns of specific amounts show this element to be practically constant it seems likely that all of the individuals regenerate as much as possible soon after the operation and then the process stops until the molt occurs.

To further illustrate the insufficiency of such a method of calculation we may consider the line of averages at the foot of Zeleny's table 6. The specific amount of regeneration for series A averages 0.444 and for series B (the ones with greater injury) 0.435 (practically equal), while the average specific rate of regeneration for A is 0.0049, and for B almost twice as much, 0.008. The columns including the number of days before the molts are not averaged, but if one will make the calculation for the 14

cases in series A, he will find the average period before molting to be 97.43 days, while for the 13 individuals of series B it is only 60.77 days. If now the average molting period of series A be multiplied by its specific rate of regeneration, we have $97.43 \times 0.0049 = 0.4774$, and a similar calculation for series B gives $60.77 \times 0.008 = 0.4861$. The two products differ but slightly. Thus the average specific rates of regeneration for the two series are to one another as the average times between the operation and the first molts. The average line of Zeleny's table 7 gives a similar result ($0.42 = 0.633$), but not so close as the above, where the two series have almost the same number of individuals, 13 and 14; in Zeleny's table 7 series A contains 14 and B 20 individuals. The only legitimate conclusion to be drawn from such figures seems to be that *Cambarus* regenerates a given amount (the specific amount of regeneration) and stops until a molt occurs; if the molt comes early, then the animal has an opportunity to continue its regeneration; and so it may be said that a crayfish which molts oftener will regenerate a limb sooner than one molting less often. Thus if greater injury causes the crayfish to molt more promptly, they grow a limb sooner merely on account of having more opportunity to grow, as the result of frequent molts, but whether the specific rate of regeneration is more in those with the greater degree of injury is not definitely shown.

Zeleny states: "The more rapid regeneration of the limbs may be the cause of the acceleration of the molting, or the opposite may be the case, or finally the two phenomena may be coördinate and only indirectly related." In the crayfish it seems that the greater injury is accompanied by more rapid molts, but I see no proof that the latter response results from a more rapid regeneration. It may be due to the regeneration taking place from a greater area.¹

Of special interest in this connection are the results of Emmel (1907) on the lobster. Only an abstract of Dr. Emmel's paper has yet been published. He has written me, however, that the specific amount of regeneration at the time of the first molt was fairly constant, and this is the important point for comparison with Zeleny's results. Emmel found that those individuals with the greater degree of injury molted slower, while those less injured molted faster. The response is opposite to that of the crayfish. The significant point is his conclusion that the rate of regeneration was slower in the more injured series and faster in those less injured—again an opposite conclusion from that of Zeleny.

If Emmel's observations that the lobsters most injured molt slower than those less injured is correct, then his second conclusion of slower regeneration from those most injured is clear. In the lobster, as in the crayfish, the regenerating bud grows as long as possible and then is pre-

¹ Emmel (1906) has shown that it is the process of regeneration itself which affects the rate of molting in the lobster.

vented from going further by the hard chitinous shell; this amount of growth (specific amount of regeneration) may very probably be attained some time before the ecdysis. Now, if we follow Zeleny's method of first calculating the specific amount of regeneration and then dividing this by the number of days elapsing between the operation and the molt, we get a slow rate of regeneration for those most injured, since they molt slower and give a larger number of days as a divisor with the specific amount of regeneration as a constant dividend. With such a method of calculation it is not a question of regeneration rate at all, but merely a consideration of the molting period. Emmel states that the later the mutilation is made in the molting cycle the more rapid is the rate of ensuing regeneration. This suggests that the regenerating bud may grow to its limit within the encasing wall in a very short time, and when the molt is long postponed it must remain quiescent for a long period.

I have entered into this somewhat detailed criticism of the work on crustacea, since it seems to me that in considering the rate of regeneration one finds himself on rather uncertain grounds when using an animal on which the continuous growth of the regenerating part can not be observed. On the other hand, *Cassiopea* is well suited to such study, since all of its eight mouth-arms are similar and the regenerating buds from the stumps of the arms grow continuously and may be constantly observed and measured.

Sixteen healthy individuals were selected and their disks carefully measured. They were arranged in eight series of two individuals each, having one mouth-arm removed from each of the first pair, two arms from the second, and so on to the seventh pair, where seven mouth-arms were removed from each. The eighth pair had four alternate arms removed. After 4 days none showed any marked indications of regenerating buds. Two weeks after the operation distinct buds were regenerating from the cut arm-stumps, though at this time it was almost impossible to determine whether there was any difference in rate. Later, however, differences in rate became evident.

By referring to table 2 comparisons may be readily made between the average specific amounts of regeneration from those medusæ with a few mouth-arms removed and those with many. The first column indicates the number of arms removed from the individual; the next column gives the diameter of each disk at the time of the operation; the third column gives the specific amounts of regeneration for the medusæ 20 days after the operation. This specific amount of regeneration is the quotient obtained when the average length of the regenerating buds from the stumps of the several arms is divided by the aboral diameter of the medusa disk. The fourth, sixth, and eighth columns show the diameters of the medusæ at intervals during the experiment. It will be noted that the animals were

gradually decreasing in size, probably due to an insufficient food supply. It should be recalled, however, that Morgan has shown for a number of forms that starvation or lack of food does not affect the rate of differentiation in the regenerating processes. Further, all animals in my experiments were under identical conditions, so that they are to be compared without regard to food supply. The fifth, seventh, and ninth columns give the specific amounts of regeneration at the times indicated. In these tables it is unnecessary to calculate a specific rate of regeneration per day, since the growth was continuous and each column of specific amounts is given for a certain number of days; obviously the specific amounts divided by the same number of days are to one another as their quotients would be. Thus the first column of specific amounts not only indicates the specific amounts of regeneration for each individual up to that time, but also the relative rates of growth during the 20 days.

At 20 days after the operation one individual with only one mouth-arm removed and both medusæ with two arms removed have regenerated faster than any of those with four or five removed arms. The ones having lost six and seven arms show faster regeneration than any others of the series. These four medusæ are, however, the smallest individuals, and young small medusæ usually regenerate faster than larger ones, even under the same conditions. The medusæ with four alternate arms removed are going at about the same rate as the two above with four adjacently cut arms. All medusæ in the table, with the exception of the last two, had consecutive or adjacent arms removed.

After 23 days the medusæ have decreased somewhat in size, as is shown by comparing the second and fourth columns. At this time, July 6, the fifth column, specific amount, shows a sudden jump when compared with the third column. This is not an actual jump occurring within the three days' time between the calculations, but is only apparent, since the specific amounts after 20 days were calculated on the basis of the original diameters of the medusæ and the calculations at 23 days are made on the diameters at this time.¹ The two columns are not to be compared. The other columns to the right are open to comparison, since the diameters were remeasured each time. The specific rates of regeneration for the individuals with one, two, and three arms removed are about the same. Those with four removed arms have a slight advantage over those having lost five arms; it must be mentioned, however, that one individual with five arms removed has failed to produce a bud from one stump and consequently its average regeneration is abnormally low. Again the ones with seven cut arms are regenerating fastest of all, and those that lost six follow next.

On the 18th of July, 35 days after the operation, the regenerating buds

¹Recent studies show that only the original diameters are to be used in such calculations, as final diameters vary with different extents of injury.

were all growing at a healthy rate, as is readily seen by comparing the specific amounts of regeneration with those of July 6. Individuals with two, three, four, and five arms cut away are going at similar rates, although showing irregular fluctuations. A point of some importance is that there is a greater difference in regeneration rates between the two individuals with three arms cut away than between any others of the series having lost from two to five mouth-arms. Those with six and seven removed arms still lead in the rate of regeneration. The two with four alternate arms cut away are going at about the same rate as the two with four consecutive mouth-arms removed.

The experiment was closed after 38 days, at which time the longest buds were slightly less than 9 mm. in length. These were produced by the medusæ in which four and five arms had been removed, which were among the largest individuals of the series. Those with one and two cut arms were regenerating at about equal rates. The two individuals with three removed arms showed a great difference between their average regeneration rates. Those with four arms removed were regenerating faster than those that had five arms cut away. Those with six and seven cut arms led the series, but, as mentioned above, these were the smallest individuals. The two with four alternate arms removed were perceptibly behind those with the four adjacent ones cut away.

TABLE 2.—Average specific amounts of regeneration from medusæ with their mouth-arms removed.

June 13.		July 3.		July 6.		July 18.		July 21.	
No. of arms removed.	Diam-eter.	Specific amt. of regeneration.	Diam-eter.	Specific amt. of regeneration.	Diam-eter.	Specific amt. of regeneration.	Diam-eter.	Specific amt. of regeneration.	
1	3.5	.0289	3.37	.0492	2.87	.0869	2.75	.0909	
1	3.5	.0357	2.75	.0454	2.37	.0526	2.25	.06	
2	3.25	.0387	2.75	.0567	2.25	.0833	2.19	.0857	
2	3.25	.0387	2.75	.0426	2.25	.0694	2.19	.0714	
3	3.37	.037	2.56	.0469	2.25	.1111	2.06	.126	
3	3.37	.037	2.75	.0418	2.5	.05	2.37	.0526	
4	3.62	.0344	3.12	.0685	2.5	.079	2.62	.1025	
4	3.62	.0344	2.75	.056	2.37	.1084	2.37	.132	
5	4.12	.029	3.5	.0608	2.87	.0956	3	.1	
5	4.12	.029	3.5	†.0351	2.69	†.0604	2.87	†.0652	
6	2.5	.052	2.19	.0765	1.75	.0997	1.87	.1116	
6	2.5	.05	1.93	.0893	1.62	.1346	1.69	.1659	
7	2.25	.0833	1.43	.1304	1.16	.1643	1.19	.1583	
7	2	.0937	1.60	.0953	1.25	.1500	1.31	.1664	
*4	3.5	.0328	3.5	†.0357	3	†.0416	2.93	†.0372	
*4	3.5	.0388	3.5	.0625	3	.0833	3	.0885	

* Four alternate mouth-arms were removed, in all others adjacent arms were cut away.
† No regeneration at all from one arm-stump.

Table 2, as a whole, indicates a condition of individual variation and fluctuation in the regeneration rates rather than anything else. It should

be mentioned that these calculations have been made, according to the usual custom, from linear measurements only, but it must be borne in mind that the actual volume of new tissue might oftentimes be more in a short stocky bud than in a longer slender one. Some of the regenerating arm-buds are short and branching, while others are long and simple in structure.

There is no doubt that the individuals with six and seven removed arms, those of the greatest degree of injury, regenerated at a faster average rate than the other medusæ of the series. It should be remembered that these were the smallest, and, as a matter of fact, in all of the experiments the small young medusæ showed more ability to regenerate rapidly than did larger old ones. Unfortunately this point was not controlled, although indirectly it is checked by a comparison with the other members of the series which are practically of a common size. In the last column, for instance, those medusæ with three and four removed arms are regenerating at a better rate than those having lost five arms, and they are also slightly smaller than the latter, although they were all of practically the same size.

The objection to this experiment, which has probably suggested itself before this time, is that too few individuals were employed. This objection is not as serious as it may seem at first sight. If among animals any such general law of regeneration exists as that the greater the degree of injury the faster the rate of regeneration, it should at least manifest itself to an evident degree. It is not necessary to use more than half as many medusæ to satisfy one's self that the rate of degeneration is fastest from the middle of a straight-cut surface and slowest from its outer corners; that regeneration is faster from a level 20 mm. from the disk margin than from any level less than that distance from the margin; that regeneration takes place fastest from the widest end of a peripheral bias-cut strip of the medusa disk and slowest from the narrow end of the strip; or, that a number of other conditions will follow, depending upon the manner in which the disk is cut. These are all facts in regeneration, and a number of them are shown by distantly related animals; and, if the degree of injury determines the rate of regeneration to any significant extent, it seems to the writer that it should manifest this determination in a more evident manner.

A serious objection to the view of a connection between the degree of injury and the rate of regeneration is the fact that from a single medusa the several arm-buds grow out at rates differing as widely as the average rates of individuals with different degrees of injury. It is difficult to understand how such a fact can be reconciled to the degree of injury idea. When one individual has six arms cut away, each at the same distance from its base, then it would be expected that all of the arms should regenerate at almost the same rate, for there is little doubt that the degree of injury is the same in each case and the surrounding conditions are as near as possible identical, being similar places on the body of one individual.

TABLE 3.—*Specific rates of regeneration from each stump of two removed arms on the same individual.*

Individual.	July 6, 23 days.			July 18.			July 21, 38 days.		
	Diam-eter.	Length of arm-buds.	Specific amt. of regenera-tion.	Diam-eter.	Length of arm-buds.	Specific amt. of regenera-tion.	Diam-eter.	Length of arm-buds.	Specific amt. of regenera-tion.
A.....	2.75	0.125	0.0454	2.25	0.125	0.0555	2.187	0.125	0.0571
	2.75	.187	.0681	2.25	.25	.1109	2.187	.25	.1142
B.....	2.75	.115	.0418	2.25	.125	.0555	2.187	.125	.0571
	2.75	.12	.0436	2.25	.187	.0833	2.187	.187	.0857

Table 3 gives the histories of each arm in two individuals which had had two of their mouth-arms cut away. The individual A shows after 23 days a greater variation between the rates of regeneration from its two arm-stumps than is shown in table 2 between the average rates of all the medusæ with one, two, three, five, and four (alternate) arms removed. At the thirty-fifth and thirty-eighth days one of the arms has grown twice as fast as the other. The B individual of table 3 also shows a distinct difference in regeneration rates between the two arms.

TABLE 4.—*Specific rate of regeneration from each stump of four removed arms on the same individual.*

Individual.	July 6, 23 days.			July 18.			July 21, 38 days.		
	Diam-eter.	Length of arm-buds.	Specific amt. of regenera-tion.	Diam-eter.	Length of arm-buds.	Specific amt. of regenera-tion.	Diam-eter.	Length of arm-buds.	Specific amt. of regenera-tion.
A.....	3.125	0.187	0.06	2.5	0.156	0.0625	2.62	0.187	0.0714
	3.125	.188	.0603	2.5	.187	.075	2.62	.264	.1006
	3.125	.23	.0736	2.5	.196	.0785	2.62	.312	.119
	3.125	.25	.08	2.5	.25	.1	2.62	.312	.119
	2.75	.126	.0458	2.37	.224	.0945	2.37	.305	.1284
B.....	2.75	.136	.0496	2.37	.241	.1013	2.37	.308	.1297
	2.75	.166	.0604	2.37	.252	.1062	2.37	.311	.1311
	2.75	.187	.0681	2.37	.312	.1315	2.37	.33	.1389

Table 4 gives the histories of each of four arms in two individuals, A and B. Here again one finds as much variation in the regeneration rates as is shown between the average rates for individuals with different degrees of injury in table 2.

Table 5 records the rates for each of the six regenerating arms in two individuals, A and B. The individual A shows a variation in rate of regeneration between the first two arms in the table and the last one which is greater than the difference in average rates of regeneration between almost any of those variously injured medusæ given in table 2. The individual B of table 5 also shows a variation in the rates of regeneration from its several arm-stumps.

TABLE 5.—Specific rate of regeneration from each stump of six removed arms on the same individual.

Individual.	July 6, 23 days.			July 18.			July 21, 38 days.		
	Diameter.	Length of arm-buds.	Specific amt. of regeneration.	Diameter.	Length of arm-buds.	Specific amt. of regeneration.	Diameter.	Length of arm-buds.	Specific amt. of regeneration.
A.....	2.187	0.1249	0.0571	1.75	0.125	0.0714	1.87	0.125	0.0666
	2.187	.1297	.0593	1.75	.139	.0792	1.87	.135	.0718
	2.187	.1875	.0857	1.75	.153	.0873	1.87	.187	.1
	2.187	.1875	.0857	1.75	.187	.1071	1.87	.253	.1343
	2.187	.1875	.0857	1.75	.193	.1103	1.87	.262	.1397
	2.187	.1875	.0857	1.75	.25	.1428	1.87	.313	.1671
B.....	1.94	.122	.0627	1.63	.125	.0769	1.69	.189	.1121
	1.94	.167	.0862	1.63	.187	.1153	1.69	.25	.1481
	1.94	.187	.0967	1.63	.25	.1537	1.69	.303	.1794
	1.94	.187	.0967	1.63	.25	.1537	1.69	.31	.1836
	1.94	.187	.0967	1.63	.25	.1537	1.69	.312	.1851
	1.94	.187	.0967	1.63	.25	.1537	1.69	.315	.1869

With such remarkable variations existing within the same individual in the regeneration rates of its arms, it is dangerous to draw conclusions from the differences shown in rate of regeneration among the individuals of a small series. As pointed out above, the variations in rate between pairs of similarly injured individuals in table 2 are as great as the differences in rate between two individuals which have suffered different degrees of injury. It must be recognized, finally, that in these medusæ the individual variation in regeneration rates is sufficient to conceal a minor variation which might be due to the degree of injury, did such exist.

These tables giving the specific amount of regeneration from the arm-bases of the same individual serve, at least, to indicate that no deductions can be made from table 2 regarding the specific amounts of regeneration from medusæ injured to greater or less degrees. Tables 3, 4, and 5 indicate still further that unless the differences in regeneration rates among animals injured to greater and less degrees are constant and marked they may very likely be accidental, or else due to the peculiar responses of that given form upon which the experiment was conducted. It is of interest to note that Scott's (1907) study of regeneration in the fish's fin and the present data from the medusæ both indicate that the extent of injury is negative in its influence on the rate of regeneration. Zeleny and Emmel's results on animals that must molt in order that the regenerating bud may continue to grow are due, I believe, to the influence of the regenerating tissue on the molting cycle.

THE INFLUENCES OF CHANGED CHEMICAL CONDITIONS ON THE RATE OF REGENERATION.

At present scarcely anything has been ascertained as to the effects produced upon regenerating tissues by changes in their chemical environments. Loeb (1904) found that *Tubularia* in a solution slightly below the concentration of sea-water would regenerate more rapidly than in normal sea-water. It was also necessary to have the solution slightly alkaline, in order to obtain a maximum growth. Loeb also made some observations on the influence of the oxygen supply and found, as in the case of embryonic growth, that an insufficient amount of oxygen retarded the rate of regeneration. A series of experiments that I (1906, 1907a, 1907b) have conducted on the developing fish egg has shown that although in a new medium the embryo may often develop to all appearances in a normal manner, yet its rate of growth is usually affected.

In the following experiments the four elements Na, K, Ca, and Mg were employed, as they seem so essential to marine life, and also form a group, as it were, the members of which act against one another or fit together in ways so as to produce a favorable balance for the maintenance of life-processes.

Mayer has performed a number of most instructive experiments illustrating the influences of these elements upon the rhythmical pulsations of *Cassiopea*. He found that sodium stimulates slightly the muscular activity and that it is "the chief stimulant of sea-water." A combination of Na, K, and Ca is, however, a greater stimulant. Magnesium must be present in order to hold such a combination in check and thus sustain a rhythmical action. Normal medusæ are but little affected by an excess of NaCl in sea-water and will pulsate for more than 18 hours in sea-water plus 1 per cent excess NaCl. A 1.55 per cent excess of NaCl was found to give rapid pulsations and to shrivel the medusa disk. A relative excess of K and Ca retards pulsation, even though the actual amount of K and Ca is that contained in sea-water.¹

Potassium temporarily stimulates and then retards pulsation; in excess it is quite poisonous. The disk comes to rest expanded, with the mouth-arms contracted. In a 0.125 per cent excess of K_2SO_4 the rate of pulsation is reduced to half the normal after 13 hours, while 1.55 per cent excess will stop the pulsations within 4 minutes.

Calcium itself is not necessary for pulsation, although by its presence the inhibiting effect of Mg is counteracted. After one has inhibited pulsation in a given manner it may be restored in solutions lacking Ca, showing that this element is unessential for rhythmical contraction. A 1 per cent sea-water solution of $CaCl_2$ reduces the rate of pulsation; recovery is immediate in sea-water.

¹ By sea-water Mayer means Van't Hoff's artificial sea-water and the retardation which he attributes to Ca and K in this sentence may in reality be due to the smaller relative amount of Na present in the solution.

Magnesium salts in sea-water retard pulsation and reduce its rate, amplitude, and energy. The disk will pulsate at twice its normal rate in sea-water minus magnesium. In a 1.6 per cent sea-water solution of $MgCl_2$ the disk pulsates slowly for half an hour and then stops.

The medusæ in the following regeneration experiments were subjected to the influences of sea-water concentrated to two-thirds and three-fourths of its original volume; or to sea-water diluted with distilled water, two parts of sea-water to one of distilled and one of sea-water to one of distilled; or to sea-water solutions of $NaCl$ $\frac{m}{12}$, $\frac{m}{15}$, $\frac{m}{30}$, and $\frac{m}{50}$; KCl $\frac{m}{40}$, $\frac{m}{50}$, $\frac{m}{75}$ and $\frac{m}{100}$; $CaCl_2$ $\frac{m}{20}$, $\frac{m}{50}$, and $\frac{m}{100}$ and $MgCl_2$ $\frac{m}{20}$, $\frac{m}{40}$, and $\frac{m}{80}$. The medusæ were all cut as shown in fig. 29. The margin of the removed piece included four of the peripheral sense-organs and was made as near as possible of the same proportional size in the several medusæ.¹

The regeneration of these medusæ is shown in tables 6 and 7, which have been arranged from two experiments run at different times. In the first column of these tables the solutions employed are listed; the second and fifth columns contain the diameters of the individuals at the times indicated, the third and sixth columns give the exact amounts of regeneration that have taken place in a radial direction from the middle of the cut surfaces; the fourth and seventh columns show the specific amounts of regeneration from the different individuals. These last amounts may be compared also as specific rates of regeneration per day, since all have consumed like periods of time in the regeneration process.

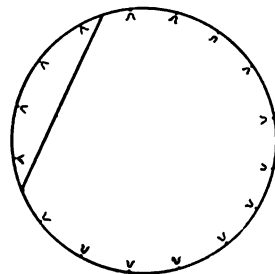


FIG. 29.—Diagram of disk with straight cut removing four of its marginal sense-organs.

The rates of pulsation of the medusæ disks were retarded in all of the solutions after the first several hours, the retardation being more marked in some cases than in others. The pulsations were always, however, slower than the controls.

The animals in the sea-water concentrated to two-thirds its original volume died within two days. Those in the three-fourths concentrated sea-water were so weakened that they showed no beginning of regeneration and were placed in normal sea-water after the third day. The diluted sea-water solutions also had a decidedly depressing effect. All medusæ in such

¹ Those in table 6 treated with KCl $\frac{m}{50}$ and $MgCl_2$ $\frac{m}{40}$ were small individuals, as indicated, and were cut in the dovetailed way shown in figure 10. This no doubt accounts in part for their apparently rapid regeneration, for it will be recalled that the deeper portion of a cut of this shape regenerates at a faster rate than a straight cut, such as that in figure 29.

media regenerated at a slow rate and threw out masses of slimy material, a reaction commonly shown when this animal is placed in disagreeable surroundings. It is recognized that the concentration and dilution of these sea-water preparations are very great and with slight concentration or dilution such injurious effects might by no means follow. The one object of the powerful solutions was to ascertain whether this animal was very resistant to changes in the osmotic pressure conditions, and the fact that they existed in these solutions for such long periods of time indicates that they are not particularly sensitive to osmotic changes.

TABLE 6.—Rates of regeneration in strange chemical environments.

Solutions.	July 4. Experiment 6 days old.			July 6.		
	Diameter.	Length of new tissue.	Specific amt. of regeneration.	Diameter.	Length of new tissue.	Specific amt. of regeneration.
Control.....	2.75	0.155	0.0568	2.56	0.25	0.0975
NaCl $\frac{m}{12}$	2.25	.093	*.0416	----	----	(†)
KCl $\frac{m}{40}$	1.87	.048	.0249	1.75	.063	.0357
KCl $\frac{m}{80}$	0.81	.093	‡.1153	0.75	.063	‡.0833
KCl $\frac{m}{100}$	2.37	.125	.0526	1.31	.129	.099
CaCl ₂ $\frac{m}{200}$	1.62	None	-----	1.5	.031	** .0208
CaCl ₂ $\frac{m}{50}$	1.13	None	-----	1.06	.063	†† .0588
CaCl ₂ $\frac{m}{100}$	2.62	0.146	0.0595	2.25	.187	.0833
MgCl ₂ $\frac{m}{20}$	2	.071	.0469	1.87	.125	.0666
MgCl ₂ $\frac{m}{40}$	1.25	.093	‡.075	1.19	.125	‡.1052
MgCl ₂ $\frac{m}{80}$	2.75	.093	.034	2.75	.16	.0568

* Back to sea-water.

† Dead.

‡ The cut on this individual is differently shaped, see the text.

§ The CaCl₂ caused a slight precipitate to form in the sea-water solutions.

|| Tetanus, put into sea-water.

** In sea-water.

†† Put into sea-water.

A close study of the tables indicates that NaCl slightly retards the regeneration rate, but the weakened appearance of the disk in the strengths of this medium used suggests that the retardation is not a direct effect of the Na, but more probably due to the animal's loss of tone.

The stronger solutions of KCl also retard regeneration. The weaker solutions, on the other hand, seem to accelerate the process so that the specific amount of regeneration from individuals subjected to their action is slightly greater than that of the control medusæ.

The medusæ in the KCl solutions have their disks fully expanded, but their mouth-arms are contracted and balled up in the center.

The CaCl₂ solutions produce a tendency toward tetanic contractions and in the stronger solutions the oral surface of the disk is often torn by this violently contracted condition. Mayer (1906) has called attention to this effect of CaCl₂ on *Cassiopea*. The rate of regeneration in all of the CaCl₂ solutions was slower than normal.

In table 7 the weaker $MgCl_2$ solutions seem to have caused the regeneration processes to proceed at a rate well ahead of the control. In table 6, however, the indicated effect is not so favorable.

TABLE 7.—Rates of regeneration in strange chemical environments.

Solutions.	July 19. Experiment 9 days old.			July 21, 11 days old.		
	Diameter.	Length of new tissue.	Specific amt. of regeneration.	Diameter.	Length of new tissue.	Specific amt. of regeneration.
Control.....	2.5	0.187	0.075	2.5	0.188	0.075
Sea-water concn. $\frac{1}{4}$	2.25	.125	*.0555	2.28	.187	†.0821
Sea-water diluted $\frac{1}{2}$	2.44	.093	.0384	2.44	.093	.0384
Sea-water diluted $\frac{1}{4}$	3	.031	‡.0104	3	.062	§.0208
$NaCl \frac{m}{15}$	2.31	.095	.0405	2.44	.125	.0512
$NaCl \frac{m}{80}$	2.13	.125	.0583	2.13	.13	.0583
$NaCl \frac{m}{50}$	2.31	.125	.054	2.25	.16	.0694
$KCl \frac{m}{50}$	2.06	.031	.0151	2	.063	.0312
$KCl \frac{m}{75}$	2.12	.142	.0661	2.25	.16	.0694
$KCl \frac{m}{100}$	1.94	.125	.0645	1.87	.187	.1
$MgCl_2 \frac{m}{50}$	1.87	.031	.0166	1.75	---	(**)
$MgCl_2 \frac{m}{40}$	1.69	.125	.074	1.69	.187	.111
$MgCl_2 \frac{m}{80}$	2.37	.187	.0789	2.31	.25	.1085

* In normal sea-water third day.

† In sea-water and regenerating very rapidly.

‡ Medusa throws out slime.

§ Ceased to secrete the slime.

** Disk inverted and new tissue torn away.

These experiments show, on the whole, no marked indication of differences in the specific actions of the several ions upon the rates of regeneration. The experiments are few, but even so they indicate the very complex nature of growth and regeneration processes which are dependent upon so many secondary influences and interactions that it will be found difficult to determine when an element is producing a direct effect upon the regeneration rate or whether the effect is due in some indirect way to other conditions incited by the actions of the chemical used. At any rate, there is evident need for an extensive and careful study along these lines with the view of analyzing, as far as possible, the relationships between regeneration rate and definite osmotic and chemical changes.

SUMMARY AND CONCLUSIONS.

I. When a peripheral ring of tissue is removed from the disk of *Cassiopea* the cut margin of the disk promptly begins to regenerate a new rim. The rate at which the new tissue is formed depends upon the width of the removed ring. The wider the ring is radially, or, in other words, the nearer the cut is made to the disk center, the faster will the resulting regeneration take place. Cuts made deep into the body of the disk regenerate tissue which increases rapidly in radial width for about ten or twelve days and then almost ceases to grow in width and begins to thicken until the new tissue is as thick as the medusa disk at the given level. The cut periphery from which only a narrow ring of tissue has been removed regenerates slowly, but almost continuously, as this portion of the disk is thin and only a slight subsequent thickening is necessary.

A small medusa regenerates proportionately faster than a larger one.

These facts are closely similar to those observed by Morgan on the earthworm, fish, and salamander. The result is interesting in that it shows that animals so distinctly different as a medusa and a vertebrate regenerate new tissues at rates which differ with different levels of the body, and that as in the process of embryonic growth the nearer the normal body size and form is approached the slower will be the rate of regeneration.

The disks cut nearest the center are injured to the greatest degree, and they might be expected to regenerate new tissue at a faster rate than those cut further from the center or less injured, if the condition is parallel to the removal of more parts. It so happens, however, that the difference in level and the degree of injury often coincide. These two factors were contrasted in other experiments, which seemed to indicate that the level of the cut was the more important in regulating the regeneration rate of the new tissue.

II. *Cassiopea* regenerates new tissue from the wounded edges of straight cuts, and "partial cut surfaces" in exactly the same manner as Morgan found regeneration to take place from similar cuts made on the fins of fishes. Such a fact is of importance; first, since it shows the same principles in regeneration to apply to the proliferation of new tissue from the appendages and the true body-surface of animals. Second, it indicates that a common principle or law regulating the rates of regeneration from different parts of variously shaped cut surfaces runs through the animal kingdom, since forms at almost opposite ends of the series, the fish and the medusa, regenerate in the same manner.

The outer corners of cut surfaces seem to exert retarding influences upon the rate of regeneration at all levels. At the inner corners of "partial cut surfaces" regeneration proceeds at a faster rate than on the straight surface. This fact is probably due to a summation of regeneration which takes place from the two sides forming the angle of the inner corner. (See figs. 13, 15, and 17.)

III. Experiments were performed to test the rate of regeneration after removal of different-sized pieces of oral epithelium at the same distance from the center and pieces of the same size at different distances from the disk center. My interpretation of Morgan's idea of "pressure" in regeneration would lead one to expect greater pressure near the center and, therefore, new epithelium should cover the more central wounds of equal size sooner than it does those more peripherally located. The experiments were not entirely satisfactory on account of a tendency of the wounds to contract while healing or regenerating new tissue. Those nearest the disk center seem to contract most, so that the result is difficult to interpret. It was noted, however, that in the circular wounds the regenerating film was widest toward the disk center, as if tissue was being proliferated out from that direction at a faster rate than from any other. (See fig. 24.)

IV. When a medusa disk is cut so that a strip wide at one end and narrow at the other is removed from the entire periphery (fig. 25) regeneration will occur along the cut edge of the strip and also from the cut margin of the remaining disk center (figs. 25, 26, and 27). In such a preparation the strip has had most body-tissue removed from its narrow end; also it is least injured at the broad end, where least tissue has been removed. The disk center is most injured where it was deepest cut or at that place from which the wide end of the strip came and least injured on that portion from which the narrow end was cut.

The rate of generation from the strip, which when straightened would form a long triangular body, is fastest at the wide end and is gradually slower as the narrow end is reached. *It is, therefore, fastest from the part from which the least tissue has been removed.* The rate of regeneration from the disk portion is more rapid from the deep-cut part and becomes slower as the cut approaches the region of the former margin; therefore, *the regeneration rate here is fastest from the portion from which most tissue has been removed.* In both cases it will be observed that it is fastest at the deepest or same level, and slower as the level nears the margin. It is important to note that regeneration in both directions—toward the periphery and toward the disk center—proceeds at almost the same rate from the same level.

This experiment may be interpreted as contrasting the influences due to the degree of injury and those exerted at different levels of the animal's disk-shaped body. The level at which the cut is made is shown to be the more important factor of the two, and if the amount of injury exerts any influence on the rate of regeneration it is probably of secondary importance. One could scarcely claim that the narrow and wide ends of the strip were to be compared with two animals from which many and few appendages had been removed.

V. A ring 18 or 20 mm. in width or wider may be cut from the peri-

phery of a medusa disk and will regenerate from its cut edge until the central space is grown over with new tissue. Such a ring freshly cut had the sense-organs removed from half of its periphery, while equal-sized pieces of tissue between the sense-organs were removed from the other half, thus making the degree of injury equal on the two halves. The oral epithelium was then lightly scraped across between the sense-organ half and the other without sense-organs. After the last operation the first half continued to pulsate, while the latter came to rest, since the stimulus for pulsation seems to be derived from the sense-organs and can not be transmitted across the scraped epithelium (see fig. 28). This ring regenerates tissue toward the center until the space is covered over. The rate of regeneration from the half at rest and the half in motion is on comparisons with the controls found to be the same. The results with this medusa show that activity or effort is not capable of accelerating the regeneration rate, as authors have held to be the case in other animals. This is the most decisive experiment that I know of as a direct test of the influences of action and rest on the rate of regeneration from tissues under as nearly as possible identical conditions, being similar united portions of one individual.

VI. Medusæ having one or more of their mouth-arms removed regenerate these mouth-arms at irregular rates which are not closely associated with the number of arms cut away, or, in other words, with the degree of injury. Two medusæ, each having three of the eight mouth-arms removed, may show a greater difference between their average specific rates of regeneration than would be found to exist among the average specific rates of regeneration from individuals with one, two, four, or five mouth-arms cut away. (See table 2.) An individual from which several mouth-arms have been removed in as near as possible similar ways will exhibit as great a degree of variation among the specific regeneration rates of its several arms as will be found to exist among the average specific regeneration rates of many individuals, each having had a different number of arms removed. (Compare tables 3, 4, and 5 with table 2).

Cassiopea is well fitted for experiments of this nature, since the regenerating buds of the mouth-arms grow continuously and may be measured and compared at any time during the experiments. It is of advantage also to have the several mouth-arms almost identical in size and form, as a comparison of the regenerative processes from the individual arms of a single medusa is thus facilitated. Most of the experimental investigations pertaining to the question of the relation between the degree of injury and the rate of regeneration have been conducted on crustaceans. These animals must molt before the regenerating bud can be observed, and since the length of the molting period varies so widely among the individuals the true specific rate of regeneration is difficult to estimate. The regenerating bud very probably grows as much as the confining chitinous covering will

permit, just as the crustacean's body increases in size until its inelastic case will allow it to become no larger, and must then remain quiescent until the molting time arrives, which may be weeks or even months distant. There is also a great individual difference between the crustacean's appendages, which renders difficult a comparison of the regeneration rates from the different limb-stumps of the same specimen. *Cassiopea* is free from such objections, but it shows so much variation in the specific rates of regeneration from different individuals that it would be difficult as well as misleading to claim from the data, now at hand, that any relationship existed between the degree of injury an individual had sustained and its specific rate of regeneration.

VII. *Cassiopea* seems to be resistant to slight changes in osmotic pressure, as is indicated by its condition in concentrated and diluted sea-water.

(a) Regeneration from the disk of *Cassiopea* was slightly retarded in sea-water to which NaCl had been added. This retardation may possibly be due to the direct action of the Na ion, although considering the weakened condition of the medusæ in such solutions they might be expected to regenerate slower than the normal.

(b) Strong solutions of KCl also retard the rate of regeneration, but weaker solutions seem to accelerate the process.

(c) Solutions of CaCl_2 in sea-water have a tendency to cause muscular tetanus, the oral surface of the disk often tearing as a result of the violently contracted condition. The rate of regeneration in all CaCl_2 solutions was very slow, in some scarcely any regeneration taking place for a number of days.

(d) Magnesium chlorid in sea-water solutions exerts a rather indifferent influence over the rate of regeneration.

The chemical experiments show no marked indication of a difference in the specific action of the several ions upon the rate of regeneration. Even though such a specific influence of these ions does exist, it would be difficult to discover, owing to the complex nature of the processes regulating growth and regeneration. There is evident need of extensive and careful study along these lines.

LITERATURE CITED.

- CHILD, C. M.
 1904a. Studies on regulation. IV. Some experimental modifications of form-regulation in *Leptoplana*. Jour. Exp. Zool., I, pp. 95-133.
 1904b. Studies on regulation. V. The relation between the central nervous system and regeneration in *Leptoplana*: Posterior regeneration. Jour. Exp. Zool., I, pp. 463-512.
- EMMEL, V. E.
 1906. The relation of regeneration to the molting process in the lobster. Thirty-sixth annual report of inland fisheries of Rhode Island.
 1907. Relation between regeneration, the degree of injury, and moulting in young lobsters. Science, n.s., xxv, p. 785.
- LOEB, J.
 1904. . Über den Einfluss der Hydroxyl und Wasserstoffionen auf die Regeneration und das Wachstum der Turbellarien. Arch. f. d. ges. Physiol., 101 H., 78, pp. 340-348.
- HARGITT, C. W.
 1899. Experimental studies upon *Hydromedusæ*. Biol. Bulletin I, pp. 35-51.
- KING, H. D.
 1898. Regeneration in *Asterias vulgaris*. Arch. Entwickl-Mech., VII, pp. 351-363.
- MAYER, A. G.
 1906. Rhythmical pulsation in *Scyphomedusæ*. Carnegie Inst. Washington Pub. 47, pp. 1-62.
- MORGAN, T. H.
 1902. Further experiments on the regeneration of the tail in fishes. Arch. Entwickl-Mech., XIV, pp. 539-561.
 1906. The physiology of regeneration. Jour. Exp. Zool., III, pp. 457-500.
 1907a. Regeneration. German translation, Moszkowski. Leipzig.
 1907b. Experimental Zoölogy, New York.
- PRZIBRAM, H.
 1901. Experimentelle Studien über Regeneration. Arch. Entwickl-Mech., XI, pp. 321-345.
- SCOTT, G. G.
 1907. Further notes on the regeneration of the fins of *Fundulus heteroclitus*. Biol. Bulletin XII, pp. 385-400.
- STOCKARD, C. R.
 1906. The development of *Fundulus heteroclitus* in solutions of lithium chlorid, with appendix on its development in fresh-water. Jour. Exp. Zool., III, pp. 99-120.
 1907a. The artificial production of a single median cyclopean eye in the fish embryo by means of sea-water solutions of magnesium chlorid. Archiv. Entwickl-Mech., XXIII, pp. 249-258.
 1907b. The influence of external factors, chemical and physical, on the development of *Fundulus heteroclitus*. Jour. Exp. Zool., IV, pp. 165-201.
 1907c. Preliminary report upon regeneration in *Cassiopea xamachana*. Year-Book Carnegie Institution of Washington for 1907, No. 6, pp. 118-119.
- WILSON, E. B.
 1903. Notes on the reversal of asymmetry in the regeneration of the chelæ in *Alpheus heterochelis*. Biol. Bulletin IV, 1902-03, pp. 197-210.
- ZELENY, C.
 1903. A study of the rate of regeneration of the arms in the brittle-star, *Ophioglypha lacertosa*. Biol. Bulletin VI, pp. 12-17.
 1905a. Compensatory Regulation, Jour. Exp. Zool., II, pp. 1-102.
 1905b. The relation of the degree of injury to the rate of regeneration. Jour. Exp. Zool., II, pp. 347-369.
 1907. The effect of degree of injury, successive injury, and functional activity upon regeneration in the scyphomedusan *Cassiopea xamachana*. Jour. Exp. Zool., V, pp. 265-274.

APPENDIX.

After the present paper had gone to press Dr. Zeleny published the results of a study on the effect of degree of injury, successive injury, and functional activity upon regeneration in the scyphomedusan *Cassiopea xamachana*.¹ In this paper he concludes that "removal of six of the eight oral arms constitutes the most favorable degree of injury for the regeneration of each arm, and that from this optimum there is a decrease in both directions." The data, however, on which this statement depends is not altogether conclusive. One finds on studying his table that the extremes of variation in regenerative rates among similarly injured individuals are in the large majority of cases greater than the differences between the average specific amounts of regeneration for two groups of individuals injured to different degrees. With such a wide range of variability shown among the few individuals one is uncertain as to the real significance of the table. Nevertheless, the data do seem to show a steady advance in specific rates of regeneration up to a maximum where six arms were removed.

In a general way my results on regeneration of the oral arms might also be interpreted, like Zeleny's averages, to show a gradual increase in regenerative rates with an increase in degree of injury. Zeleny seems inclined to emphasize the importance of this apparent increase in regenerative rates, while I believe the great range of variability in the regenerative rates shown by the small number of individuals studied should not be overlooked and that it renders a general conclusion from such observations very uncertain.

Zeleny's study of the effect of the rhythmical pulsation of the medusa disk on the rate of regeneration may be compared with my experiments bearing upon the same subject. Our results agree and both indicate, contrary to the view of some observers, that functional activity is negative in its influence on the rate of regeneration. Zeleny compared the regenerating margins and centers of medusæ disks pulsating rhythmically with those from other medusæ at rest, and found that in four of the six pairs of cases the non-pulsating individuals regenerated faster than the pulsating ones. In the fifth pair the two were equal, and in the sixth the pulsating individual regenerated faster.

The question might arise whether in my own experiments the influence from the pulsating half may not be conveyed to the resting half. Improbable as is such a view in itself, the interpretation is negated by my con-

¹ Journal of Experimental Zoölogy, v, 2, pp. 265-274. 1907.

trols, some of which, as in Zeleny's experiments, consisted of an entire pulsating ring and others of the entire ring at rest.

The level at which the margin is removed determines the rate of regeneration from the cut surface, and this possibility is not mentioned in Zeleny's account. It is difficult to remove a marginal strip of exactly the same proportions from several medusæ, even when careful measurements are made. Slight differences in the depths of the cuts would easily account for the results, but since the rates of regeneration from both the center and outer margin are considered by Zeleny this is in a manner checked. In my "ring preparations" described above, the level of the cut is controlled as far as possible, since the disk center also forms the center of the circular mass of tissue that is removed. Thus the cut surface and the disk periphery are concentric circles; therefore, all places on the cut surface are at a common level. My observations are aided by the fact that the pulsations were continuous throughout the experiment, while Zeleny's rhythmic pulsations always stopped before the end of his experiment.

THE DEVELOPMENT OF ARTIFICIALLY PRODUCED CYCLOPEAN FISH—"THE MAGNESIUM EMBRYO"

BY

CHARLES R. STOCKARD

WITH ONE PLATE AND SIXTY-THREE TEXT FIGURES

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INTRODUCTION

Development is the resultant of the interaction between the inherent tendencies contained within the egg substance itself and the external conditions which surround and act upon this substance. The usual interaction of these factors gives rise to normal animal forms. When, however, either factor is changed an unusual form results; in the one case there arises a germinal variant, and in the other an anomaly occurs as a response to the strange external environment. The product of development, the formed animal, is then to a certain extent a creature of its environment. On the other hand the importance of the internal factors must be recognized although modern experimental work often-

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times points in a direction which would indicate that these factors may be largely modified in their influences by the external conditions.

Most monstrosities or abnormalities in development are due to the action of external factors, either mechanical, as pressure, or chemical. Mammals, birds and reptiles, with their complex embryonic membranes, offer many opportunities for the production of secondary abnormalities arising from unfavorable mechanical or physical conditions. Foetal amputations and scars, membrane fusions distorting facial development, and many other such deformities are in most cases probably due to secondary influences on development. Besides these there are deformities of a different nature, such as the excessive monsters, *monstra in excessu*, in which certain organs have over developed or produced supernumerary parts; and contrasted with them are defective monsters which fail to complete themselves and are therefore less than a normal individual. It is with this latter class, *monstra in defectu*, that the present study is concerned. These defective individuals may be grouped into two sub-classes: first those in which certain organs fail to complete themselves, as in cleft palate, hare-lip, arrests in the development of the heart and other parts of the circulatory system. Second, individuals in which certain paired organs occur singly or without mates. True Cyclocephali or cyclops monsters find their place in this last group.

Cyclops monsters have long been known to occur in man and other mammals and are described in many of the earliest medical works. In these beings the one eye is in the middle line of the face and often shows external evidence of a double composition. The nose which normally arises above the eyes and grows down between them as the face develops is here mechanically prevented from descending by the presence of the median eye in its path. The foetus, therefore, has a proboscis-like nose above the eye. The brain and other parts of the body are sometimes deformed though they may be normal.

Among the lower vertebrates true cyclops monsters have been recorded by Spemann ('04) as resulting from mechanical injuries to the eggs of the amphibian, *Triton taeniatus*. These mon-

sters were double-headed with one or both heads showing the cyclopean defect and were not of the usual single cyclopean type found in man and other mammals.

Two years ago (1907) I carried out experiments in which I was able to produce typical single cyclopean fish. This was the first record of the occurrence of cyclopia among fishes. It is also the first case of consistently producing vertebrate monsters such as are known in nature by changing the chemical environment in which the eggs develop. These embryos are in main details similar to the mammalian cyclops, having a single median eye and anteriorly placed double nasal pits.

The monsters were produced by allowing the eggs to develop in sea-water in which there was an excess of $MgCl_2$. Cyclopia occurred in a large percentage (at times 50 per cent) of the embryos. The discovery was made so late in the spawning season that it was impossible to investigate the details of the cyclopean defect or rear the embryos to hatching in order to observe their ability to swim or to see. The method of production, however, offered such an exceptional opportunity to obtain abundant material for studying all stages of development and degrees of cyclopia that this more extended survey was undertaken.

The following account includes a comparative study of cyclopean embryos from the earliest appearance of the optic vesicle to the perfectly formed free-swimming fish with a functional cyclopean eye.

The experiments were conducted in the Marine Biological Laboratory at Woods Holl, Mass., during the past summer, while occupying one of the rooms of the Wistar Institute.

MATERIAL AND METHOD.

As in my previous experiments, the eggs used were those of the teleost fish, *Fundulus heteroclitus*.

The method of producing the defect was much the same as that previously employed although expanded and modified in many ways. During the early part of the season it was difficult to find

solutions of the proper strengths and the eggs were either killed or unaffected. After a few experiments, however, a strength of MgCl_2 in sea-water was found that gave a large percentage of cyclopia, in many cases again causing 50 per cent of the eggs to form such individuals. This was a $\frac{1}{80}$ M solution prepared as follows: 19 cc. of a molecular solution of MgCl_2 in distilled water was added to 41 cc. of sea-water. This is not then an actual $\frac{1}{80}$ M MgCl_2 solution but it is $\frac{1}{80}$ parts molecular MgCl_2 . Making the solution in this way adds to the sea-water, water lacking all of its constituents except the Mg and thus increases in a greater proportion the excess of Mg present.

Cyclopia occurred in a series of similarly prepared solutions ranging as follows: $\frac{1}{80}$ M, $\frac{1}{60}$ M, $\frac{1}{40}$ M, $\frac{1}{30}$ M, $\frac{2}{30}$ M, $\frac{2}{20}$ M and $\frac{2}{10}$ M MgCl_2 . A point of importance is that the proportion of cyclops embryos produced gradually rises in this series up to the $\frac{1}{30}$ M solution and then falls off again. To illustrate concretely, in Experiment VII the $\frac{1}{80}$ M solution caused 12 per cent of the eggs to form cyclopean embryos, the $\frac{1}{60}$ M gave 30 per cent, the $\frac{1}{40}$ M 22 per cent, while $\frac{1}{30}$ M gave 50 per cent with the cyclopean defect. Continuing the series, the $\frac{2}{30}$ M falls off to 30 per cent and the $\frac{2}{20}$ M gives 23 per cent, while in the $\frac{2}{10}$ M no cyclopia occurred and the eggs were all killed. It must be born in mind that these percentages are for the eggs that formed embryos and not for the total number of eggs first put into the solution. The peculiar fact is, that in a series of MgCl_2 solutions we reach a place where a maximum number of cyclopean embryos occur and in strengths both weaker and stronger than this the number of cyclopean individuals is less. If the defect is due to osmotic pressure, we should not expect a greater pressure to bring about a more normal development. If the action is chemical, we do not usually reach a chemically effective dose and find that a greater dose is less effective. It might be argued that below the point of maximum occurrence of the cyclopean defect, the solutions are insufficient to effect any but the weaker embryos, so that a small number of cyclops appear; above this point the solutions are so strong that all except the hardest embryos die in early stages and those surviving are so resistant that only a few give the cyclopean defect.

At the maximum point the normal or ordinary individuals, which predominate, would be affected, and here the greatest number of cyclopean embryos occur.

As I previously mentioned, the MgCl_2 is found to be rather toxic to these eggs during the earlier stages of development. Many die at this time, but in the medium strength solutions 70 to 80 per cent live and form embryos and in the weaker solutions often more than 90 per cent live. After the early embryo is formed, however, the high death rate falls and a dead embryo is of rare occurrence in any of the solutions. Many embryos were kept in the solutions thirty days and some hatched in strengths as strong as $\frac{1}{80}$ M. If, on the other hand, the eggs are removed from the solutions when sixty or seventy hours old, when the cyclopean condition is readily distinguishable, and placed in sea-water they grow much better and many hatch normally. Some of the cyclopean fish came out on the twelfth day after fertilization, though usually they were much slower in emerging. The control embryos hatch in from eleven to twenty days, depending chiefly upon the temperature.

Solutions of MgCl_2 in Distilled Water

Distilled water solutions of MgCl_2 of several strengths; $\frac{1}{80}$ M, $\frac{1}{160}$ M, $\frac{1}{320}$ M, $\frac{1}{640}$ M and $\frac{1}{1280}$ M were not effective. The eggs either died during early stages or developed into embryos with two normal eyes. I had found (1906) that salts of lithium induce the same typical defects in *Fundulus* eggs in both sea-water and distilled water solutions. Such solutions have opposite conditions of pressure, being in one case hypertonic and in the other hypotonic and thus remove all question of osmotic effects as a cause. It was hoped that Mg might also act in the two solutions which would have made it certain that the direct action of the magnesium ion is responsible for the cyclopean condition of these embryos. The problem of cyclopean formation seems, however, to be more complex. It involves the action of magnesium in the presence of certain or all of the sea-water salts.

Solutions of $MgSO_4$ and $Mg(NO_3)_2$ in Sea-water

Sea-water solutions of $MgSO_4$ prepared in a similar manner to the $MgCl_2$ solutions above were employed. The following strengths $\frac{1}{8}$ M, $\frac{1}{10}$ M, $\frac{1}{12}$ M, $\frac{1}{15}$ M, $\frac{1}{20}$ M, $\frac{1}{25}$ M, $\frac{2}{30}$ M, $\frac{2}{40}$ M, $\frac{2}{50}$ M, $\frac{2}{60}$ M, $\frac{2}{70}$ M, and $\frac{2}{80}$ M were ineffective, the eggs in these solutions developing normally with very few deaths at any stage.

$Mg(NO_3)_2$ solutions in sea-water caused typical cyclopia indistinguishable in all respects from that produced in $MgCl_2$. The following strengths were used: $\frac{1}{8}$ M, $\frac{1}{10}$ M, $\frac{1}{12}$ M, $\frac{1}{15}$ M, $\frac{1}{20}$ M, $\frac{1}{25}$ M, $\frac{1}{30}$ M, $\frac{1}{40}$ M, and $\frac{2}{50}$ M. These $Mg(NO_3)_2$ solutions also killed many embryos during the early stages of development. Cyclopia occurred in from 4 per cent to 40 per cent of the eggs in $\frac{2}{50}$ M, $\frac{1}{20}$ M, $\frac{1}{25}$ M, $\frac{1}{30}$ M, and $\frac{1}{40}$ M. These strengths are comparable to those most effective for $MgCl_2$, both as to the amount of magnesium present and as to osmotic pressure.

Mixtures of $MgCl_2$ + $NaCl$; $MgSO_4$ + $NaCl$; and $Mg(NO_3)_2$ + $NaCl$

Mixtures of $MgCl_2$ and $NaCl$ were added to sea-water as follows: 12 cc. of a molecular solution of $MgCl_2$ was added to 12 cc. of $NaCl$, and 36 cc. of sea-water was then taken to make the entire quantity up to 60 cc. This solution will be spoken of as $\frac{1}{2}$ M + $\frac{1}{2}$ M, the first term referring to the $MgCl_2$ present and the second to the $NaCl$. On this basis the following mixtures were used: $\frac{1}{2}$ M + $\frac{1}{2}$ M, $\frac{1}{4}$ M + $\frac{1}{2}$ M, $\frac{3}{10}$ M + $\frac{1}{2}$ M, $\frac{7}{30}$ M + $\frac{1}{2}$ M, in which the $MgCl_2$ was varied and the $NaCl$ kept constant, and $\frac{1}{4}$ M + $\frac{1}{4}$ M, $\frac{1}{4}$ M + $\frac{1}{2}$ M, $\frac{3}{20}$ M + $\frac{1}{2}$ M, $\frac{1}{2}$ M + $\frac{1}{2}$ M, in which the amount of $NaCl$ was varied also.

Such mixtures caused the development of cyclopia, the best results were obtained in $\frac{1}{4}$ M + $\frac{1}{2}$ M, where as times as many as 25 per cent occurred. The $\frac{3}{10}$ M + $\frac{1}{2}$ M gave in one case 30 per cent of cyclopia. The $\frac{7}{30}$ M + $\frac{1}{2}$ M gave 11 per cent. It will be seen that the amount of $MgCl_2$ present in these mixtures is less than that necessary to cause similar results when used alone. This is a peculiar fact and one for which I know of no explanation. Similar results (Stockard '07b) were found with mixtures

of salts in distilled water where the final pressure was less than that of sea-water, the normal medium of the eggs. It is also true that if such substances as the sugars be added to a salt solution, a smaller dose of the salt becomes effective in the presence of the sugar. Morgan ('06) first called attention to this peculiar fact in studying the effects of solutions upon developing frogs' eggs. This would seem to indicate that the effects were due to osmotic pressure conditions and by slightly raising the pressure with another element the effective agent was assisted in its action, but my lithium experiments (1906 and 1907b) are against such a view.

A number of mixtures of MgSO_4 and NaCl were tried, all giving negative results. Mixtures of $\text{Mg}(\text{NO}_3)_2$ and NaCl as follows were used: $\frac{1}{4} \text{ M} + \frac{1}{8} \text{ M}$, $\frac{1}{16} \text{ M} + \frac{1}{8} \text{ M}$ and $\frac{7}{80} \text{ M} + \frac{1}{8} \text{ M}$. The first two caused eggs to develop cyclopia. These are mixtures closely similar to the effective MgCl_2 and NaCl solutions.

We conclude that cyclopean monsters are produced in *Fundulus* eggs by the action of sea-water solutions of MgCl_2 , $\text{Mg}(\text{NO}_3)_2$, and mixtures of MgCl_2 and NaCl and $\text{Mg}(\text{NO}_3)_2$ and NaCl . No other solutions of the many I have tried during three summers gave similar effects. Other salt solutions and sugar solutions exerting practically the same osmotic pressure also fail to cause cyclopia.

Another argument opposed to the view that osmotic pressure is the cause is the fact that *Fundulus* embryos are so resistant to changes in pressure. Since two Mg salts give similar results when used in sea-water solutions, it seems probable that the action of Mg, either directly or indirectly, is responsible for the result. Eggs have been subjected to this action before the first cleavage, during the two-cell stage and just before going into four cells, with similar results. No attempt was made to determine at how late a stage the cyclopean condition could still be caused, though it could doubtless be induced after the eggs had passed much beyond the four cell stage. The fact is that cyclopia may be caused in an egg which has started its development normally and which would have given a two-eyed embryo. The idea of a germinal origin of the defect in this case seems excluded. Cyclopia in this instance is the result of unusual external conditions.

CYCLOCEPHALI AND "MONSTRA MONOPHTHALMICA ASYMMETRICA"

The magnesium solutions induce the formation of two distinct types of eye monstrosities. The first type is the typical cyclopean monsters, which exhibits a series of individuals showing various degrees of cyclopia. Beginning with a normal individual having eyes in their usual position, we find others in which the eyes are slightly inclined forward and somewhat closer together than usual; or the eyes are still more approximated and occupy an unusually anterior position. (See the diagram, Fig. 1). Next in the series are individuals with their eyes approximated but still distinctly separate, having two optic nerves and two eyeballs with their choroid coats in intimate approximation. We next find the true cyclopean eye which still shows a double nature having two optic nerves; the retina has a paired arrangement and either one or two lenses may occur, depending upon the degree of distinctness of the two components. This eye generally occupies a ventro-median position and looks forward, inclining slightly downward. The eye in others is completely single, showing no indication of a compound structure; it has one optic nerve, a single retinal arrangement, one lens and one pupil. This is the perfection of cyclopia and many embryos possessing such an eye are apparently normal in other respects, except the mouth and nose. They have a typically bilateral brain and are perfectly capable of free-swimming movements. Passing beyond this stage of cyclopia, we find embryos which have gone to the extreme and show only a defective antero-median eye. In some individuals the eye is represented merely by a choroid vesicle. The step beyond this is the entire absence of the eye. Diagram Fig. 1 gives a schematic illustration of the various degrees in the cyclopean series thus outlined. The histological conditions shown by such a series will be considered beyond. It is important to understand that this series is made up of different individuals showing various degrees of cyclopia and that a cyclopean monster does not pass through these steps in its development. The cyclopean defect is foreshadowed in its final condition when the optic vesicle first separates itself from the brain.

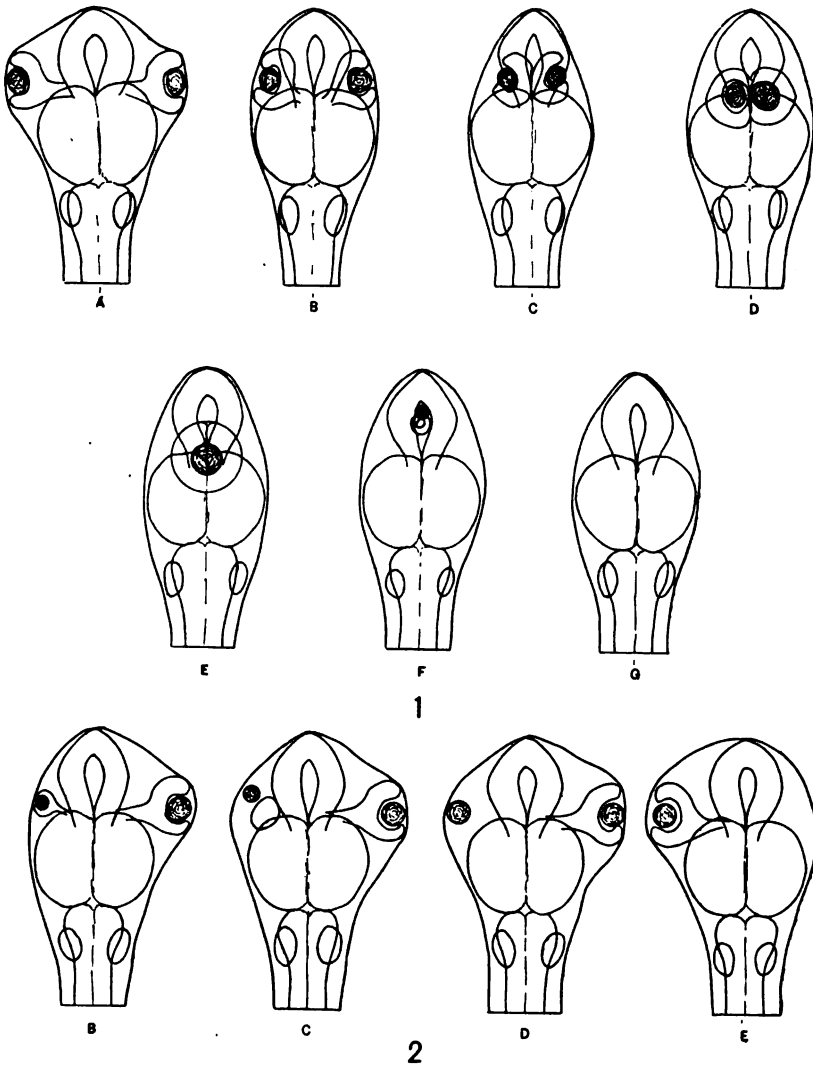


Fig. 1 Diagrams of the various conditions of the cyclopean defect as shown by the "Magnesium embryos," from the normal *A* to complete absence of the eye *G*.

Fig. 2 Diagram of the monstrum monophthalmicum asymmetricum series, from one defective eye *B* to complete absence of one eye *E*.

The second type of optic defect caused by magnesium is a new monstrosity and may be termed *Monstrum monophthalmicum asymmetricum*, the monster with one asymmetrical eye. It has only one perfect eye which represents one of the normal pair and occupies the usual lateral position. This eye is in all cases perfect while its mate may be indicated by either a small eye, by a mere cellular mass representing an optic cup, or all indications of the second optic cup may be wanting. (See Fig. 2.) This peculiar one-eyed condition exists in many of the embryos in the magnesium solutions. Had such a defect resulted from a mechanical operation, it would probably have been interpreted to mean that one eye anlage was injured and the other not. With the solutions, however, we get a clear case of the gradual dropping out of one eye by comparing different individuals, and here as in cyclopia the defect is present from the earliest appearance of the eye, and is not due to a gradual degeneration, or arrest during development. A study of sections of these embryos makes the conditions clearer.

a The Living Cyclopean Embryos from the First Indication of the Defect to the Time of Hatching

The optic vesicles appear in most eggs when about thirty hours old; at this time the blastopore is just closing and the embryo is well mapped out on the embryonic shield. Many attempts were made to select cyclopean individuals at this stage but it could not be done with a great degree of certainty, since some embryos are always slow in giving off the optic vesicles and these at times appear to have only one, but when examined some hours later are found to be normal. A number of eggs were selected, however, at thirty hours old which proved to be cyclopean on later examination.

At about forty hours the defect is plainly detectable so that one may arrange the eggs very accurately into two groups, the cyclopean individuals and the normal. After such a separation, none of the normal embryos ever exhibited the cyclopean defect in later stages, although kept in Mg solutions. A number of such tests as this in connection with the study of sections convinced me that

the cyclopean condition existed as such from the first appearance of the optic vesicles, and no subsequent fusion of the two optic vesicles or cups took place after that time.

A forty-two hour embryo is shown in Fig. 3. It is seen to be well formed and the optic vesicles are clearly outlined on either side of the head. Fig. 4 illustrates a cyclopean individual of the same age. The single optic vesicle occupying a ventro-median position is shown through the transparent embryo. This young individual with its newly formed optic vesicle shows a typical cyclopean condition, and no indication is seen of two separate elements that would later fuse. Other embryos at this age have abnormally twisted cephalic regions and show no indication of eyes, although the cyclopean eye might easily be concealed by the bent brain (Fig. 5). Such embryos at later periods are found to be cyclopean and to have narrow tubular brains showing more or less abnormal bendings.

When the embryos are about three days old, the brain has expanded and presents a distinctly bilateral appearance; the optic cups are well developed and the lenses are partially formed (Fig. 6). A cyclops monster at this time has a well formed body and the brain is often normal, though in Fig. 7 it is inclined toward the narrow tubular condition and is anteriorly twisted. The ventromedian eye is clearly seen through the brain and the outline of its lens is distinct. A somewhat younger, sixty-five hour, embryo is shown in Fig. 8 with a superficially perfect brain and two optic cups intimately approximated. The telencephalon is seen to protrude beyond the eyes, as is the case in the normal individual (Fig. 6).

Three four-day embryos are shown by Figs. 9, 10 and 11. The brain and spinal cord at this time are clearly mapped out by a coarse pigmentation, the two hemisphere-like portions (*corpora bigemina*) of the mid-brain are distinctly formed and the eyes are large with the lens clearly outlined within the cup. A cyclopean monster with a perfectly formed large ventro-median eye is illustrated by Fig. 10. Comparing its brain and other parts with the normal (Fig. 9), one fails to find any important deviations. The abnormal condition of the narrow tubular brained cyclops,

Camera lucida sketches of living embryos from $MgCl_2$ solutions

- Fig. 3 A normal embryo of forty-two hours, the two optic vesicles present.
- Fig. 4 A typical cyclopean individual of forty-two hours. The single median eye (*O. V.*) is represented in circular outline.
- Fig. 5 An embryo of same age, twisted brain, no optic vesicle shown.
- Fig. 6 Normal seventy-two hour embryo.
- Fig. 7 Cyclops of same age. The eye, *op.c.*, in ventro-median position.
- Fig. 8 Sixty-five hour embryo, two ventrally approximated optic cups.
- Fig. 9 Normal four day embryo—bilateral brain outlined.
- Fig. 10 Four day cyclops, large ventro-median eye and typically bilateral brain, *op.c.*, the eye.
- Fig. 11 Four day cyclops with narrow tubular central nervous system.
- Figs. 12 and 13 Five day cyclops, narrow tubular brain with waist-like constrictions dividing them into fore, mid and hind-brain regions. Ventro-median eye.
- Fig. 14 Five day cyclops with ventro-median eye and dorsally humped brain.

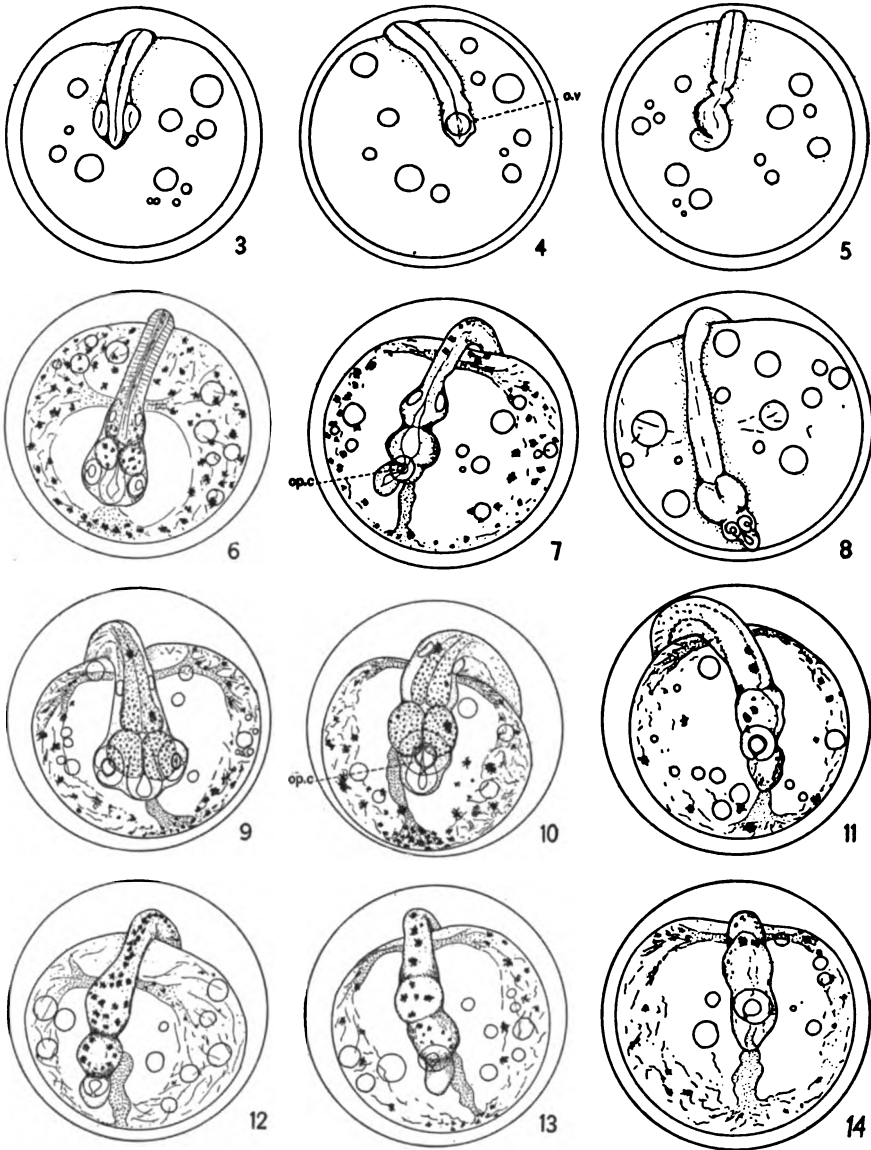


Fig. 11, is evident. Fig. 12 shows a common type of cyclopia with the three primary brain regions separated by waist-like constrictions. Two other variations of the narrow tubular condition are found in Figs. 13 and 14. The embryos are five days old and no changes of importance occur from this time until the hatching period is reached, except the usual progressive development of the eye structures.

The normal embryos generally begin hatching when about twelve days old, one cyclopean monster hatched at this time but most such individuals were much later than the normal in coming out. A twelve day cyclopean fish is seen in dorsal view in Fig. 15 and ventrally in Fig. 16. The large cyclopean eye projects forward and occupies the position usually taken by the mouth at this time. A slight indention along its mid dorsal line suggests its double nature, although the ventral view (Fig. 16) shows this same eye to possess only one pupil and lens. The brain of this specimen is practically normal. An embryo with the two eyes intimately approximated is shown in front view in Fig. 17. The eyes are joined and each looks forward in a direction slightly towards the side to which it belongs. A common variety of cyclopean fish is one in which the eye is unusually small and occupies an extremely anterior position; Fig. 18 shows such an embryo. This variety is usually unable to hatch, although a few were assisted in breaking through the membrane. They swam rather abnormally, owing to a twisted condition of the body. A dorsal and ventral view of a cyclopean fish is shown in Plate I, Figs. A and B. This indicates the striking appearance presented by these embryos.

b Free-Swimming Cyclopean Fish

Many embryos, showing the cyclopean defect in various degrees, hatched normally and were capable of swimming in a manner indistinguishable from ordinary two-eyed fish. These monsters gave many indications of ability to see. They went to the more brilliantly lighted side of the dish with the normal ones. They darted away in a normal fashion when any object was placed in front of the eye, while similar objects put at equal distances from

their tails caused no excitement. In two instances they lived for ten days, which is about as long as the two-eyed embryos can survive without food. At this time the entire content of the yolk-sac has been absorbed. The embryos in nature doubtless begin feeding previous to this stage. The cyclopean individuals appear to be as active as the normal and their ability to live would seem to depend only upon the possibility of their obtaining food.

A normal fish eight days after hatching is illustrated by Fig. 23. The mouth projects forward beyond the dorsal tip of the head and the two eyes are lateral in position. A cyclopean embryo eight days after hatching is shown in Fig. 24. Here the two eyes are united and occupy the position which the mouth has in Fig. 23. In Fig. 25 a perfectly cyclopean eye is shown in dorsal view: the same individual is seen in lateral and ventral views in Figs. 26 and 27. This fish swam in a normal manner. In the lateral position the mouth is shown projecting ventrally as a proboscis-like structure. This condition is due to the fact that the single antero-median eye occupies the position normally assumed by the mouth and thus obstructs the usual forward growth of its structures. The mouth, therefore, remains ventro-posterior to the eye and grows downward, presenting the proboscis-like appearance.

Such a condition recalls in a striking way the nose of the mammalian cyclops. In mammals the cyclopean defect is accompanied by a proboscis-like nose situated in the forehead above the median eye. The nose in normal development grows downward to its facial position, but in cyclopia the median eye obstructs its path and forces the formation of the proboscis-like organ in the forehead. The same explanation holds for the fish's mouth where the eye prevents its forward growth, producing the proboscis-like organ.

It is interesting to find that the mouth in cyclopean fish stands in a position so as to fall in the gill series as number one, all the gills and the mouth have the same general direction. I have found that in *Bdellostoma* the mouth arises in a manner similar to the gills and actually at first arches dorsally and only secondarily arches ventrally. It may have originally been a member of the gill series, as Dohrn (1875) has long thought. It would be

Camera sketches of the living embryos in magnesium solutions

Fig. 15 Dorsal view of twelve day cyclopean monster, the antero-median eye with furrow indicating its double nature.

Fig. 16 Ventral view of the same individual, the eye possesses a single pupil and lens.

Fig. 17 A twelve day embryo, ventral view showing two eyes intimately approximated.

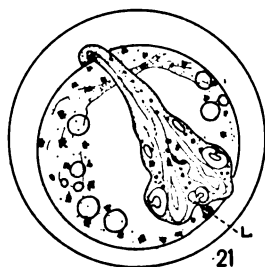
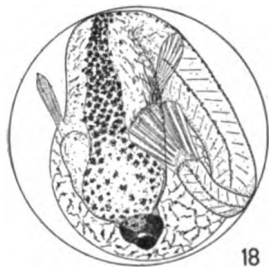
Fig. 18 Fourteen day embryo. Small extremely anterior cyclopean eye with protruding lens, extreme cyclopia.

Fig. 19 A five day *Monstrum monophthalmicum asymmetricum*; the left eye has no mate.

Fig. 20 A similar twelve day monster lacking its left eye.

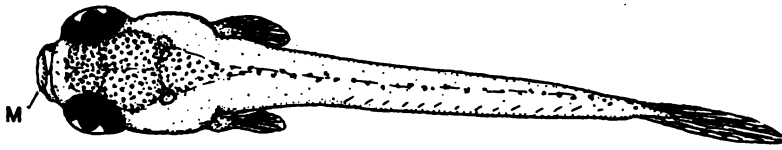
Fig. 21 An incomplete diprosopus monster seventy-two hours old. Two brains, two normal lateral eyes and one perfect middle eye, the other middle eye indicated by the circular lens *L*.

Fig. 22 The same monster when eighteen days old, three perfect eyes. The embryo hatched three hours after this drawing was made and swam abnormally.

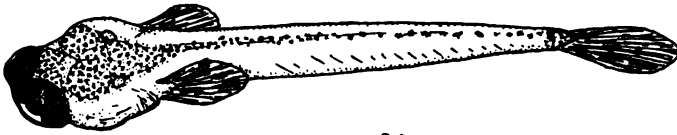


Camera sketches of free-swimming fish

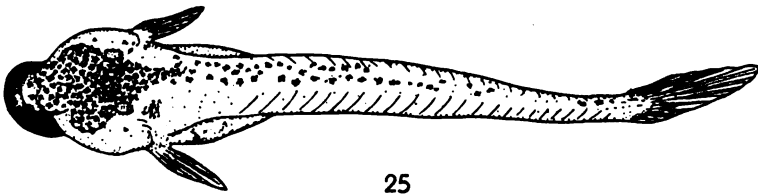
- Fig. 23 Normal individual. *M*, its anteriorly placed mouth.
- Fig. 24 Incomplete cyclops, two eyes joined and occupy the position usually taken by the mouth.
- Fig. 25 Dorsal aspect of a perfect cyclops. Antero-median single eye.
- Fig. 26 Lateral view of same. The mouth *M* is forced by the eye to remain in a ventral position and hangs down as a proboscis-like structure.
- Fig. 27 Ventral view of same fish, note perfectly single eye, one lens and one pupil, *ys.*, yolk-sac.



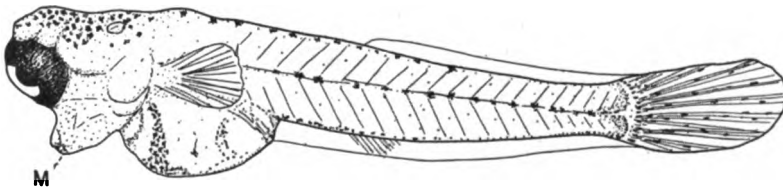
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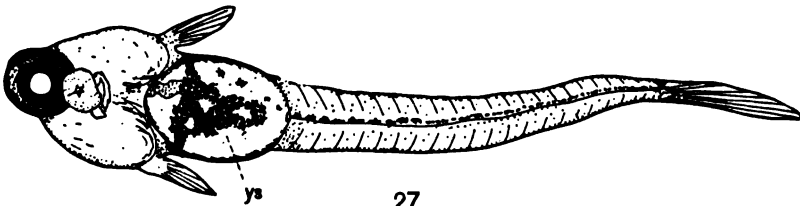
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interesting to know whether the "cyclopean mouth" is functional. The mouth does not possess a wide opening as it would normally although a small aperture is sometimes distinguishable near the end of the proboscis. No attempt was made to feed the embryos.

c Living Monstra Monophthalmica Asymmetrica

These asymmetrical one-eyed monsters may also be identified in early stages of their development. They have a single eye situated on one side of the head. Such an eye appears in some cases as though it were cyclopean and one might easily imagine the cyclopean eye becoming displaced from its usual median position to one side or the other of the head. Studying such eyes in section, however, clearly shows their single unmated origin and condition. An embryo of this kind is shown when five days old in Fig. 19. The brain is slightly abnormal and the pigmentation scarce for such a stage of development. The eye occupies the usual place of the paired eye of that side. A twelve day embryo shortly before hatching is illustrated by Fig. 20. The shape of the body and of the head is comparatively normal. The unpaired eye is slightly forward of its usual position.

Many of these embryos hatched. A few of them swam in circles, often whirling around with great rapidity, much as Japanese waltzing mice do. Others swam in irregular spirals and only progressed in a straight direction with difficulty. This peculiar one-sided manner of swimming is not due to asymmetrical vision, but results from a defective muscular arrangement, the animal's body being slightly bent or twisted so that it is unable to straighten perfectly. Some embryos with this eye on one side had normally straight bodies and these were capable of swimming in a direct course with apparently as much ease as a two-eyed fish or the symmetrical cyclopean embryos.

These monsters also lived, free-swimming, for some time. In a few cases their mouths were perfect, but in others the mouth parts were distorted or twisted by an asymmetrical condition of the ventral head region.

MORPHOLOGY OF CYCLOCEPHALIA

It was mentioned above that the optic outpushings became visible on the sides of the brain at about thirty hours after fertilization. At this time the brain of *Fundulus* is a solid mass of cells without a central ventricle. The optic bodies are not hollow at first, but are solid outpushings which later develop central cavities. The cavity generally forms in the optic outpushings while the brain is yet solid. Dareste ('91) has advanced hypothetically the idea that if the anterior vesicles of the brain did not develop, a contact would be maintained between the "parties retiniennes" up to a certain time and consequently they would unite to give a median cyclopean eye. If this were in reality the cause of cyclopia we might expect all Teleosts like *Fundulus* to be normally cyclopean since in them the eyes arise while the brain is without a ventricle. Spemann ('04) finds in cyclopean Triton embryos that although the tube is hollow, the eye anlagen are defective from the beginning. The matter of a closed brain would then seem to be unimportant in a consideration of the causes of cyclopia.

a Earliest Indication, Exact Position and Condition of the Eye

When forty-one hours old, the brain as shown in trans-section by Fig. 28, is still a solid mass. The two normal optic outpushings have developed small cavities but no indication of invagination of the vesicles or ectodermic lens structures are seen.

A section through the optic region of an apparently one-eyed monster when forty-one hours old, is shown by Fig. 29. The sections of this series show only one ventro-lateral eye vesicle. The vesicle is large and distinctly optic in nature, while on the opposite side is shown a thick cellular wall from which the brain is becoming separated. Such an individual resembles more a *Monstrum monophthalmicum asymmetricum* than it does the cyclopean type.

Fig. 30 shows a transverse section through the eye of a forty-nine hour embryo which exhibits a perfectly clear case of cyclopia. Here the brain is beginning to form a cavity and the optic vesicle with a well defined central cavity is just invaginating to form the

optic cup. This eye occupies an almost ventro-median position and is united to the brain by a solid cellular stalk. Its contact with ectoderm from which the lens will arise is not established as the head-fold does not yet extend back to this point. An eye in such a ventral position will oftentimes come in contact with the ectoderm at a later stage than would a normal lateral eye. Ordinary two-eyed individuals at this age (forty-nine hours) were, like this cyclops, just beginning to form the optic cups and the lateral ectoderm over the incipient cups showed a slight thickening, the earliest indication of a lens. As a rule the cyclopean eye is somewhat slower than the normal in its rate of development and may generally be compared with the eyes of slightly younger two-eyed individuals.

Several embryos at this age lack eyes entirely and belong to the blind variety presently to be described.

Two-eyed embryos when fifty-four hours old possess well-formed optic cups and lenses still connected with the ectoderm, although projecting into the cavity of the cup. The nasal pits are clearly marked ectodermal invaginations in an anterior and slightly median position relative to the eyes. The brain possesses a well developed central cavity. A cyclopean eye of a distinctly double composition from a fifty-four hour embryo is shown in cross-section by Fig. 31. The optic cup is bilateral and two lens anlagen are indicated by the thickened ventral ectoderm. The section of the brain dorsal to this eye is small and hollow. It is a portion of the diencephalon which is between a larger telencephalon and a much larger mid-brain. This eye would finally have produced a large median cyclopean organ of the double type with two retinal areas and two lenses. Its connection with the brain is through two closely approximated stalks and two optic nerves would probably have formed later. Comparing such an eye with that of Fig. 32, of like age we find that here the optic cup is single and one lens is forming. Both sections show the eye in practically similar positions. The embryo from which Fig. 32 was taken possesses a well formed telencephalon and two lateral nasal-pit thickenings of the anterior ectoderm.

A horizontal section of a fifty-four hour double-eyed cyclops

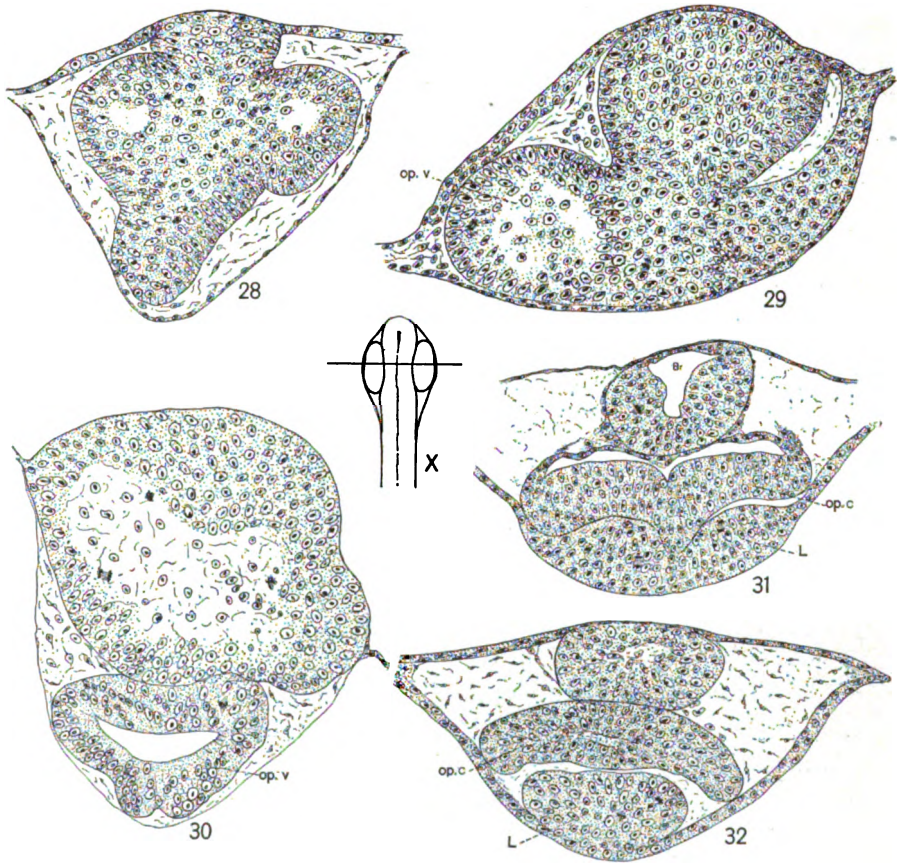


Fig. 28 A trans-section through the optic outpushings of a normal forty-one hour embryo. The brain is solid and cavities are just forming in the optic outpushings.

Fig. 29 Trans-section through the single optic vesicle (*op.v.*) of a forty-one hour embryo from $\frac{1}{3}\delta$ M MgCl_2 . The optic process is situated laterally and no indication of a like process exists on the other side.

Fig. 30 A slightly oblique section through the cyclopean optic vesicle of a forty-nine hour embryo from $\frac{1}{3}\delta$ M MgCl_2 , *op.v.* optic vesicle.

Fig. 31. Cross section through double cyclopean eye of fifty-four hour embryo from $\frac{1}{3}\delta$ M MgCl_2 , *op.c.* optic cup; *L*, lens thickening of ectoderm; *Br*, normal bilateral brain.

Fig. 32 Section of single cyclopean eye in similar embryo. *L*, lens; *op.c.* optic cup, small solid diencephalon above; *X*, guide figure indicating the plane of the sections.

is given in Fig. 33. Such a section is most instructive. The condition of the eye is much the same as that shown by the transverse section, Fig. 31. The cup is double and two ventral lenses are present. The section passes below (ventral) the diencephalon so that no part of it shows; the telencephalon is indicated in front of the eyes and a thickening of the forward ectoderm shows the nasal plate, posteriorly or behind the eyes the mid-brain is cut in horizontal section.

A sagittal section of a typical cyclopean embryo is shown by Fig. 34. Here we see the eye and the brain in the third dimension. The telencephalon in front, the diencephalon above the eye, and behind this the large mid-brain with a spacious median cavity. In front of the eye is also shown a median ectodermal thickening, the double nasal pit. The eye is single and exactly ventro-median in its position and connects in a more lateral section with the brain at about the point where the telencephalon and diencephalon join. The lens and retina are differentiating into their typical structures. One may obtain a clear mental reconstruction of the cyclops monster at this age by comparing Figs. 31, 32, 33 and 34, the transverse, horizontal and sagittal mid-planes of the cyclopean eye.

The early stages just described illustrate the cyclopean defect in its various degrees, and the eye throughout its development retains the original condition of singleness or doubleness. No evidence whatever can be found of subsequent fusions during development. Two clearly approximated eyes arise in that condition and remain so without fusing to give a double cyclopean eye, and a double eye never attains to the single condition by a more intimate union of its parts. The statement made in my (1907a) former paper, p. 257, that "the fusion of the two components may take place at different periods within a certain limit" is incorrect, as I (1908) have pointed out in a short note on the subject. This statement was one of interpretation and was based on a comparison of late embryos which showed different degrees of cyclopia. It seemed from such an incomplete study that the eyes were more or less double or compound, depending upon the stage in development at which they had become approxi-

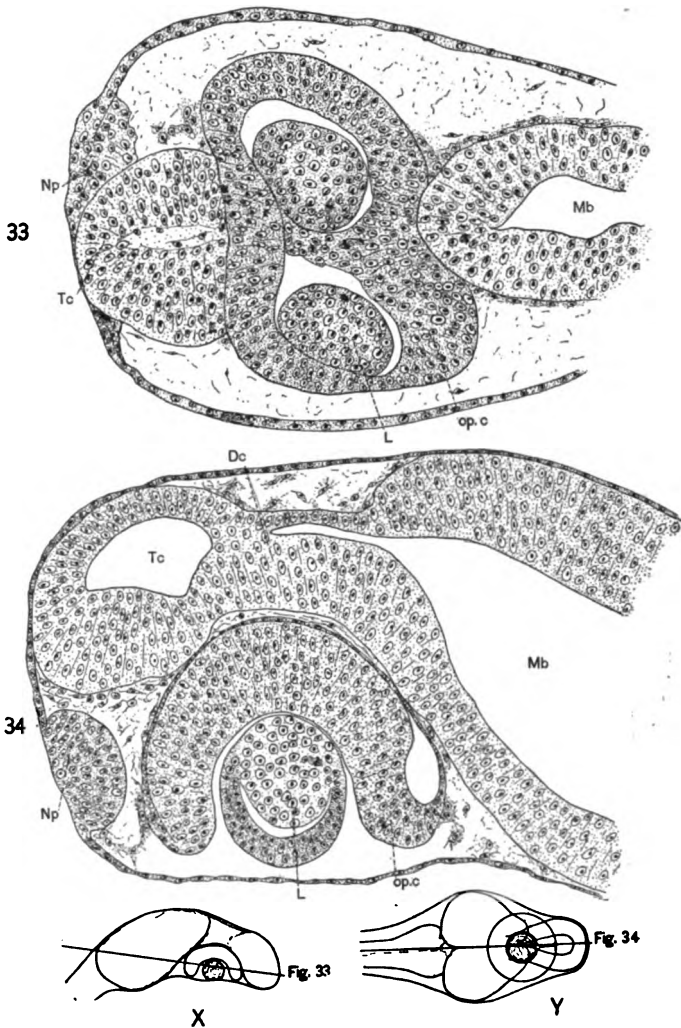


Fig. 33 Almost horizontal section through a double cyclopean eye of a fifty-four hour embryo in $\frac{1}{8}$ M MgCl₂. See guide figure X for plane of section. *Np.*, nasal plate; *L*, lens; *op.c.*, optic cup; *Tc.*, telencephalon; *Mb.*, mid-brain.

Fig. 34 Sagittal section (guide figure Y) through typical single cyclopean eye showing its ventral position below the diencephalon *Dc.* The nasal pit, *Np.* is median; *L*, lens; *op.c.* optic cup; *Tc.*, telencephalon; *Mb.*, mid-brain.

mated. The point is one which can only be proven by a number of direct observations on all ages of cyclopean embryos and careful study of sections; such a study has convinced me that no fusion of the eyes takes place after they are once clearly given out from the brain.

It seems advisable for later stages to consider groups of embryos showing various degrees of the cyclopean defect.

b Incomplete Cyclopia; Double Eyes

Under the term incomplete cyclopia may be considered individuals with eyes abnormally close together although separate. Among *Fundulus* embryos such individuals exist and a series of stages connect these embryos with those in which the two eyes are intimately connected or joined together. An individual of this kind when sectioned will show the eyes as in Fig. 35. This section is from a four day embryo, the two eyes are united in the median line of the head and both are perfect eyes with a lens, single retina and one optic nerve. The choroid coat as indicated by the heavy line is just beginning to form. Fig. 36 shows a section of two eyes which are more intimately united. This case is the common "hour-glass" eye of cyclopia. The two eyes are independent, except for their waist-like connection and each has its lens, single pupil, retina and distinct opticus. The optic nerve of the right component is seen entering the optic cross at the base of the brain. The brain in this embryo is remarkably perfect, as it is in many cyclopean monsters, and I see no reason whatever for attributing the defect to a "single brain" or any other gross malformation of the cephalic region. Many embryos with deformed brains possessed two normal eyes and the converse is true, many normal brains were accompanied by cyclopean eyes.

Leaving the "hour-glass" eye, we find the double-eye shown in Fig. 37, having a common optic chamber each half of which is supplied by one component. Two lenses and two pupils are present and generally two optic nerves, although they may run so nearly parallel that the two are difficult to distinguish. A single nasal pit is present in the embryo from which Fig. 37 is a section. All of the cyclopean monsters possess two distinct auditory vesicles.

Fig. 38 is a section through a unique double eye; no other such case was found. The two retinal components are connected along their median dorsal line within the brain and extend down facing one another. They are like the two sides of a leguminous pod; between the two a single lens is placed suggesting the seed in the pod. Enclosing the ventral part of the retinal components is a choroid coat shown in heavy black. This choroidal coat does not fully encompass the retinal areas, a part of which extends dorsally far up into the brain. The anterior end of the eye is V-shaped in section. The optic cup anlagen in this case must have been closely united from their first origin in the brain, since portions of the retinal region are still contained within the brain itself, yet during development they did not fuse into a single eye. A single nasal pit is present and the mouth is ventral and proboscis-like.

An almost single eye is indicated in section, Fig. 39. The choroid coat surrounds the retina, the latter showing slight traces of its compound nature. Two lightly staining regions of nerve tissue are seen and the entire eye is unusually wide laterally. The single lens is normal. The brain here is also normal and the eye occupies a ventro-median position. A further union of the eyes gives the

c Perfect Single Cyclopean Eye and Normal Brain

The cyclopean eyes are in many cases perfectly single, resembling in all respects, except their position, one eye of a normal pair. They are placed immediately ventral and their antero-posterior mid-plane is in the median line of the embryo. The brain in such a cyclops is often normal in all general respects. Figs. 40 and 41 represent horizontal sections through the brain regions of such a cyclopean fish when seventy-seven hours old. Fig. 40, the more dorsal section, passes through the mid-brain and shows the two lateral, hemisphere-like bodies (corpora bigemina) with well formed cavities. Behind these the section cuts the floor of the hind-brain for some distance and finally crosses it where the head bends. Passing ventrally through a number of sections, we find the one shown in Fig. 41. Here only a small ventral

Transverse sections of different degrees of double cyclopean eyes

Fig. 35 Section of eyes in four day embryo, the two eyes united. Choroid coat beginning.

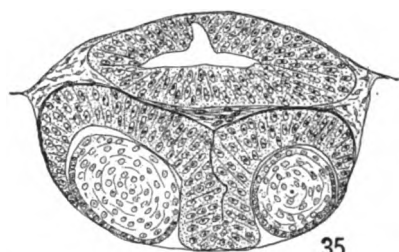
Fig. 36 Section of "hour-glass" eyes, the optic nerve of the right component entering the normally bilateral brain. From a sixteen day embryo, the retinae and lenses differentiated. *r.o.n.*, right optic nerve; *Ch.*, choroid coat.

Fig. 37 Section of eye in hatched embryo. Double-eye with two pupils and two lenses. Retina undifferentiated.

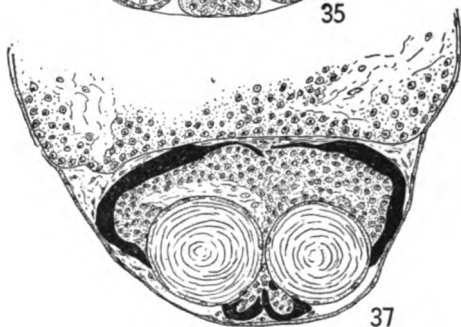
Fig. 38 Hatched cyclops, section through the peculiar eye with two components facing and lens between them (see text).

Fig. 39 Section through almost single cyclopean eye, only indication of its compound nature paired retinal arrangement. Brain normal.

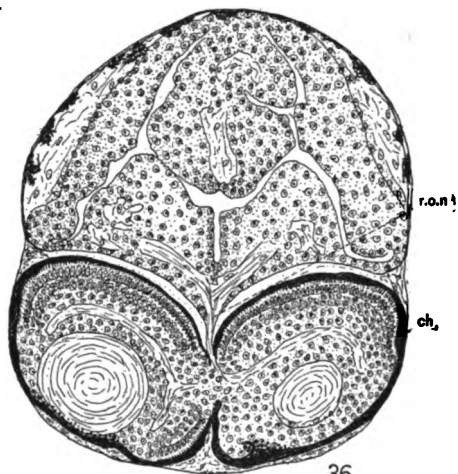
Guide figure *X* indicates the plane of all sections and the eye position in the several specimens.



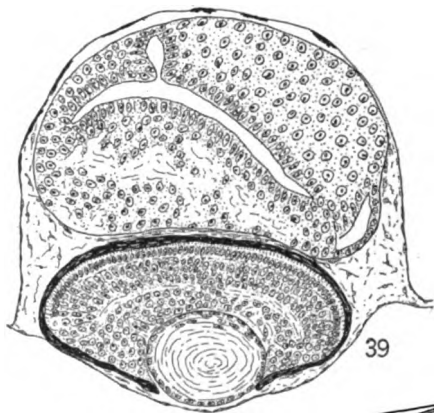
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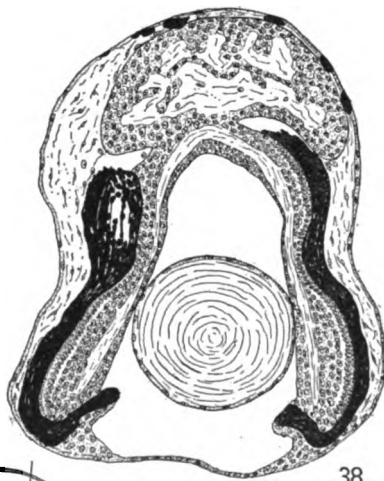
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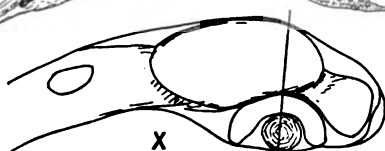
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38



X

part of one of the corpora bigemina is cut and the completely single eye with its lens is found lying ventrally and in a median position. The double olfactory pit is seen in front of the eye and somewhat to one side of the head. The posterior part of the section runs below the hind-brain and finally cuts it as the head bends just in the middle region of the well formed auditory vesicles. The section thus presents the three sense organs, the single cyclopean eye, the nasal pits united into a double pit; the paired ear vesicles alone are in their usual positions.

A transverse section through the eye of a four day embryo is illustrated in Fig. 42. The retina is unusually wide laterally but no other indication of doubleness is shown. The choroid coat is beginning to form and the eye is connected with the floor of the brain by a single cellular stalk. The retina at this age is only slightly differentiated and there is no arrangement into layers. This embryo has two distinct nasal plates. Several of the cyclopean fish show the nasal plates separate, although they are usually represented by an anterior double plate near the middle line.

A nine day embryo of which Fig. 43 represents a section through the eye has a finely developed brain, well expanded laterally and perfect in general shape and structure. The eye is completely single and the retina is partially formed into layers; the lens is almost transparent and the vitreous humor is being formed about it. The eye has all structures closely similar to those in a paired eye of this age and would doubtless have functioned had the embryo hatched. This specimen has a single nasal pit.

Another cyclops of perfect structure when studied in sections at thirteen days old showed the mouth posterior to the eye, hanging as a ventral proboscis-like mass. Two nasal plates were present and the eye was single. This eye, Fig. 44, was unusually far forward and although the retina was well differentiated into layers the humor had not perfectly formed behind the lens. The small section of the brain is shown in Fig. 44 to be bilateral and not unusual in appearance. Passing forward through the series of sections to a place where the anterior end of the cyclopean eyeball stops, a minute lens is found lying in a ventro-median position,

Fig. 45. This lens, although only nine micromillimeters in diameter, has differentiated and shows perfect lens fibers arranged in the usual concentric fashion. It has no connection whatever with the eye, nor with any part of the central nervous system. The small lens doubtless originated and differentiated its tissue in an independent manner. The independent origin and self-differentiation of lenses will be clearly shown in a following section of this paper. Fig. 45 also illustrates the two lateral nasal plates in section.

The cyclopean eye is thus seen to be at times single in nature, showing no trace of a double composition. This may be considered the climax or perfection of cyclopia, if such an expression is permissible. Eyes not completely united, or double-eyes, are the incomplete or imperfect cyclopean condition, while the single condition reduced or distorted may be termed extreme cyclopia.

d Extreme Cyclopia: From the Abnormally Small Anterior Cyclopean Eye to Entire Absence of Eyes

Many cases are found representing the condition of extreme cyclopia. They may be considered in order, beginning with the least modified. In discussing the living embryo mention was made of those with a small cyclopean eye placed far forward (Fig. 18). Sections of such eyes show them to be of a more or less imperfect nature and sometimes deeply buried in the tissues of the head. Fig. 46 shows a section through the small eye of a hatched embryo eighteen days after fertilization. This eye is placed in the extreme anterior tip of the head and the section shows on the right side pigment spots which lie on the front end of the forehead. The eye is unusually small and the living embryo was abnormal, being unable to swim directly forward. The nasal pits are united in the anterior eye region and a proboscis-like mouth is situated ventrally.

Two still more abnormal cyclopean eyes are shown in transverse section by Figs. 47 and 48, both from thirteen day embryos. In Fig. 47 the eye is close to the single olfactory pit, the retina is differentiated into layers, but the lens is larger than the optic cup so that it cannot fit completely into it. The brain of this individ-

Sections of perfectly single cyclopean eyes

Fig. 40 Horizontal section through mid-brain showing its corpora bigemina, *Cb*, and floor of hind brain, *Hb*, in ~~seventy-seven~~ hour cyclops.

Fig. 41 A more ventral section of same series, *E*, the Cyclopean eye; *ol.p.*, olfactory pits united. *Hb*, hind-brain and *Av.*, auditory vesicle; *Cb*, floor of one mid-brain lobe. Guide figure *X* gives plane of each section.

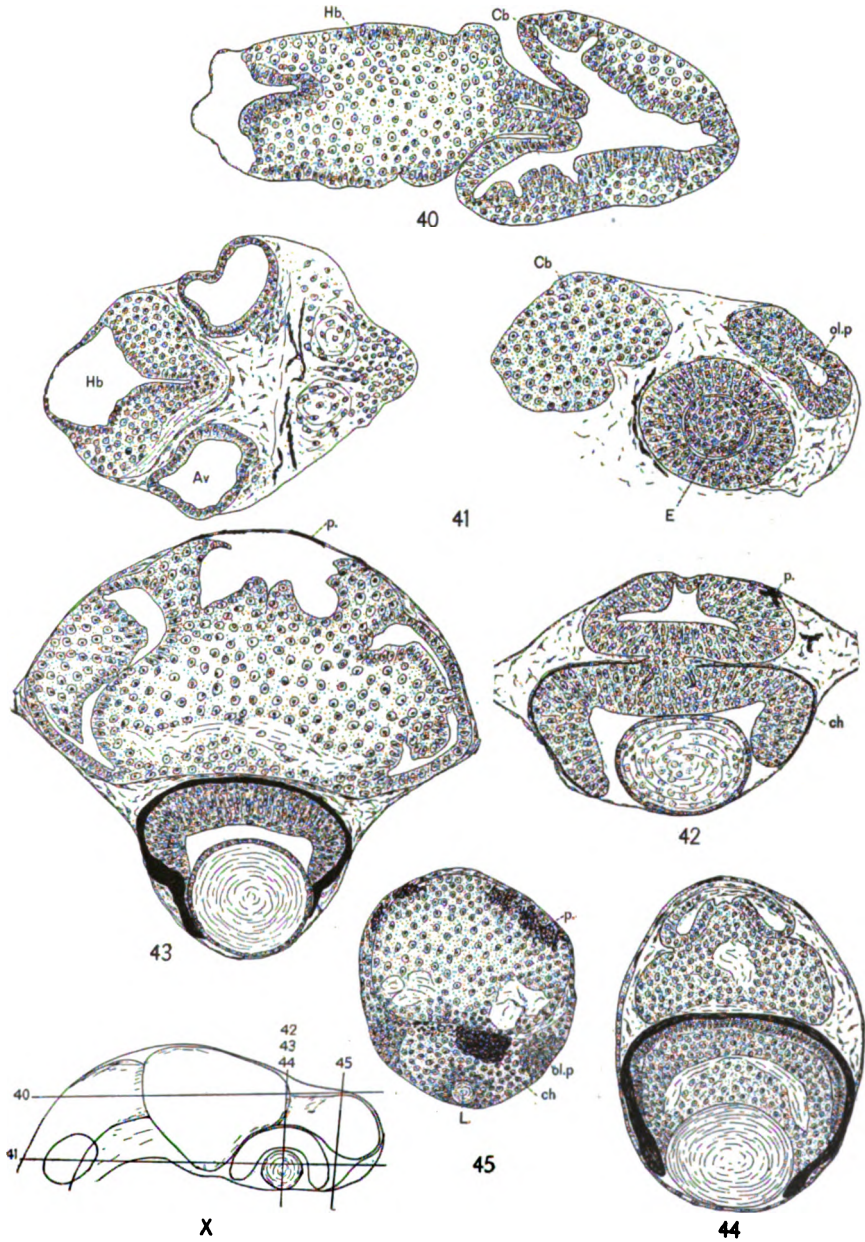
Fig. 42 Trans-section of a four day single cyclopean eye in exact ventro-median position. *ch*, choroid coat; *p*, pigment spot.

Fig. 43 Similar section of nine day eye. Humor cavity behind the lens. Note perfectly bilateral brain. *p*, pigment spot.

Fig. 44 Section of single median eye below perfectly bilateral brain, thirteen days old.

Fig. 45 A more anterior section in same series as Fig. 44. The forward tip of the eye *ch* is seen. A small lens *L* lies free near the ventral ectoderm; *ol.p.*, olfactory pit; *p*, pigment spots on anterior end of brain.

Guide figure *X* indicates plane of all sections.



ual is abnormal and the eye is out of the median line. The embryo of Fig. 48 was abnormal with the brain distorted so that the cyclopean eye was slightly to one side and far out beyond the head. The retina differentiates into layers but the lens lies out of the central position, and would be unable to function efficiently.

A peculiar condition is found in the embryo from which sections shown in Figs. 49, 50 and 51 were taken. This very small eye was again in an extremely anterior position, though almost in the median line. The lens is as large as the optic cup and protrudes far out beyond its edge. Fig. 49, the most anterior section of the three, passes through the great circle of the spherical lens and shows it entirely outside the optic cup. On passing back in the series to where the lens is less in size, we reach the anterior edge of the optic cup and choroid coat, Fig. 50. Continuing back in the series of sections, the lens disappears and the optic cup alone is shown in Fig. 51. The lens in this eye is clearly too large for the accompanying cup as was also the case with the two eyes just described. The size of these lenses is, therefore, independent of the size of the optic cup. Lewis' ('04) idea that the cup regulates the size of the lens does not apply to these embryos, nor does the rule for the amphibian that the origin of the lens is dependent upon the influence of the cup.

A step beyond this condition of a small anterior eye with its ill-fitting lens may be illustrated by an embryo in which the eye is a minute choroidal sphere buried in mesenchyme below the brain and in the median line. In life this specimen seemed entirely eyeless, but sections showed this small eye-like structure (Fig. 52) in the position typically taken by a cyclopean eye. Such cases as this emphasize the necessity of sections in order to correctly interpret the conditions of cyclopia and conclusions based only on superficial studies are necessarily unreliable. The nasal pits were in the normal lateral position. Passing back in the sections to the region usually occupied by the two eyes, it will be seen that on one side a typical lens occurs (Fig. 53). The lens is well differentiated and completely isolated from all connections with either nervous or eye tissue. A band of muscle is seen in the figure to touch the inner edge of the lens.

The occurrence of this lens recalls at once Herbst's ('01) argument regarding the independent origin of the lens. He held that "if the lens really developed independently of the optic cup, then in the case of median cyclopia the two lateral lenses should arise in their usual positions; but they do not, and furthermore, the cyclopean cup gets a lens from ectoderm out of the usual lens-forming region." The *Fundulus* embryos show lenses arising at times in their usual places and often in other places, independently of the optic cup. We may suppose that in these embryos certain areas of the ectoderm are at times out of their normal positions, and thus explain the promiscuous distribution of independent lenses.

Finally, embryos exist in which no indication of the optic cup can be found, these may be said to have passed beyond the extreme cyclopean condition. They are not ordinary individuals that are merely blind, since the mouth is usually distorted and sometimes the snout-like structure which accompanies cyclopia is present. This suggests the possibility that the "proboscis-mouth" is not entirely due to its normal position having been usurped by the cyclopean eye. Some of these embryos have free lenses and others no optic parts at all. Figs. 54 and 55 are two transverse sections from the same embryo, the anterior one shows a lens lying against the olfactory pit but free from all connection with the central nervous system. Fig. 55 shows a second lens lying close against the brain tissue. This embryo has no indication whatever of optic cups, and seemed eyeless in life. Other individuals when carefully examined in section had neither an optic cup nor any lens-like structures.

We have thus reviewed a series of forms beginning with the usual two-eyed embryos and passing through all degrees of double eyes to single cyclopean eyes, to extremely small cyclopean eyes, to individuals finally with only lenses present and no optic cups and others with neither lens nor cup.

The extreme cyclopean condition

Fig. 46 Cross-section of hatched embryo, small cyclopean eye located in anterior tip of head. The nose is anterior to this section. *p*, pigment on "forehead" of embryo.

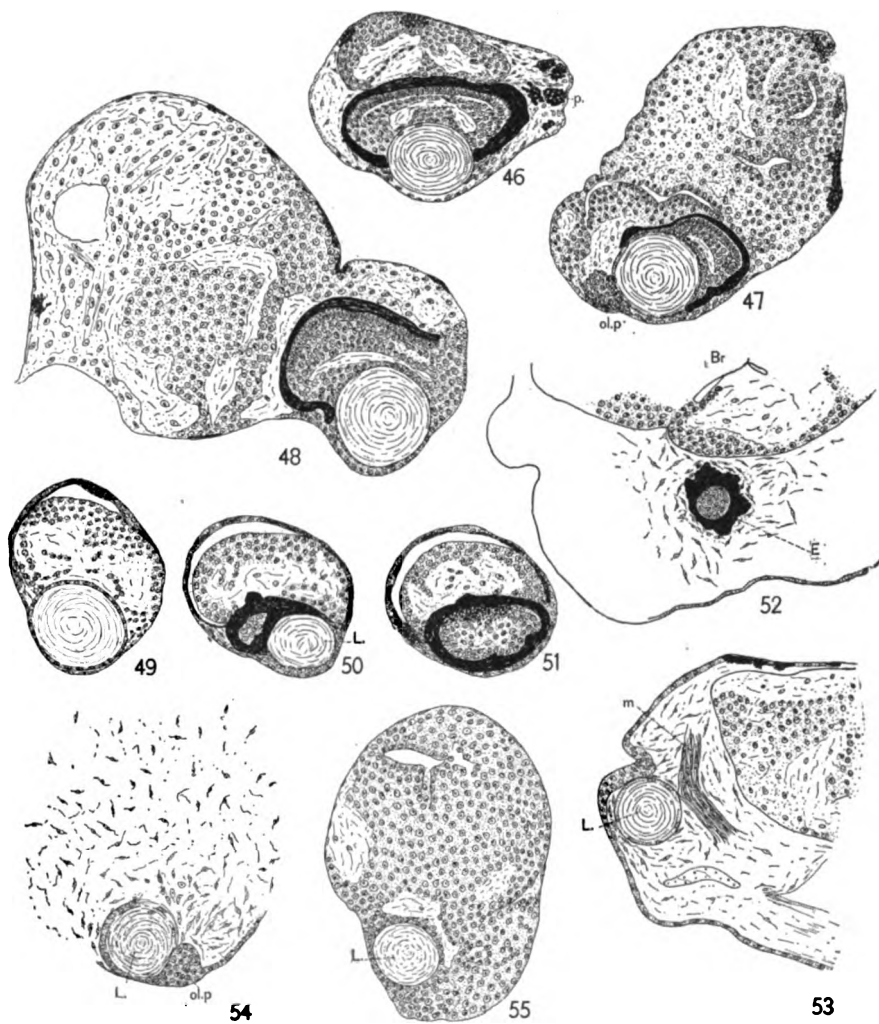
Fig. 47 Section of thirteen day embryo. Small cyclops eye with large lens, differentiated retina and abnormal brain partly surrounding the eye; *ol.p.*, nasal pit.

Fig. 48 Section of thirteen day cyclops with eye far forward and out of median line beneath an abnormal mass of the brain.

Figs. 49, 50 and 51 Sections of a small anterior cyclopean eye with large lens projecting out of optic cup. The first section Fig. 49, is most anterior, the great-circle of the spherical lens, Fig. 50, tip of lens in the edge of optic cup, and Fig. 51, center of optic cup behind the lens.

Figs. 52 and 53 Sections of thirty day embryo which seemed eyeless in life. Brain abnormal. Fig. 52, the cyclopean eye is represented by a choroid vesicle, *E*. The more posterior section, Fig. 53, shows a perfect lens *L*, in the usual lateral position, but no optic cup exists. A band of muscle *m* is between the lens and brain.

Figs. 54 and 55 Sections of two lenses *L*, one forward by the olfactory pit, *ol.p.*, the other more posterior and surrounded by brain tissue. No optic cup present in this nine day embryo.



INCOMPLETE DIPROSOPUS WITH THREE EYES AND ONE
ADDITIONAL LENS

A most valuable object for study was an incomplete diprosopus monster which appeared in my solutions. This individual had two heads separated as far as the lateral eye region. It appeared as indicated by Fig. 21 when seventy-two hours old. The two brains are separate, almost back to the auditory vesicles. Two normal eyes are shown in outer lateral positions while between the heads one eye, perfect in shape, is mated with the outer eye of the left head and a circular body occupies the usual position of left eye on the right head. The embryo seemed normal in other respects and was in a vigorous condition.

The monster when eighteen days old had developed to the usual size and was still hardy. At this time it presented a striking appearance as indicated imperfectly by Fig. 22. Three large eyes normal in form and capable of movement looked out from the double head. All visible evidence of the circular body shown near the middle eye when seventy-two hours old had disappeared. The middle eye was clearly paired with the left eye of the left head component and the right eye of the right head seemed mateless. A single pair of auditory vesicles were present. The young fish respired and twisted vigorously within the membrane. Three hours after this drawing was made, the embryo hatched and swam about in a circular fashion, the body not straightening perfectly. The free living animal was kept for five days and then preserved for sectioning.

The sections show the presence of two brains, one spinal cord and one normal mouth leading into a pharynx with its series of gills, while a second short throat is present in the right head. There are two notochords back to the middle of the yolk-sac and one from there on. The rear end of the medulla becomes single and only one pair of ear vesicles are present. There are two olfactory pits anterior and median to the lateral eyes.

Three perfectly normal eyes exist. They possess clearly differentiated retinæ, irides, humor chambers and lenses. Two of these eyes are connected in the usual way with the brain of the

left head and one with the brain of the right. Fig. 56 is a section showing the middle eye somewhat back of its center so as to bring the edges of the other eyes into the figure. The middle eye is more anterior in position than the two lateral ones, owing to the slight obliquity of the left head. A distinct lens is shown in the cup in Fig. 56. On going backwards in the series we reach a section passing through the middle of the two lateral eyes and the posterior end of the middle eye (Fig. 57). The section shows dorsally the huge double brain and ventrally a central throat and most interesting of all a fourth lens. This lens lies against the outside choroid coat of the middle eye and is in just the position (recognizing a displacement due to development of the middle eye) to be the lens of the left eye of the right head, if such an eye were present. We thus have in this double head three typical eyes and the fourth represented by a free lens. It was impossible to detect the clear lens in the living embryo which emphasizes again the necessity of sections for a definite interpretation of the conditions existing in these monsters. Conclusions drawn from observations on the living eggs without the comparison of sections may be incomplete. The sections further make clear the nature of the circular outline shown against the middle eye of the seventy-two hour embryo (Figs. 21 and 57). Comparing the figures of sections and those of the whole embryos, it will be remembered that the sides of the sections are transposed, since the drawings of the total embryos are made from a simple microscope and the sections from a compound microscope which inverts the image.

This incomplete diprosopus monster increases the series of eye monstrosities so that it passes through the cyclopean group to beyond the normal. The diagram (Fig. 58) illustrates in a simple way the various conditions we have considered and emphasizes the continuous nature of the series. Beginning at one end with eyeless individuals, we pass gradually through a series with small buried cyclopean eyes (which may be indicated in the diagram by a palpebral opening, such as similar mammalian cyclops would show), to the perfectly single cyclopean eye, to the double eye with one lens and pupil, to the hour-glass eye with two lenses and two pupils, to two independent but closely approximated eyes, next to

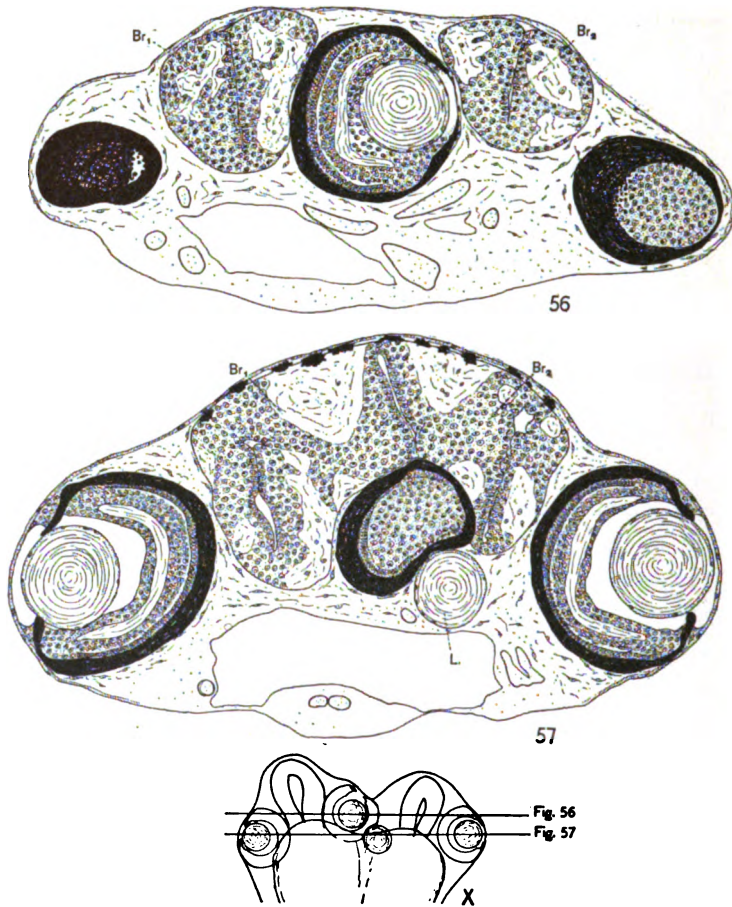


Fig. 56 Section through anterior median eye and edges of lateral eyes of hatched incomplete diprosopus; Br_1 Br_2 , the two brains

Fig. 57 More posterior section through middle of lateral eyes, posterior part of middle eye, and an additional fourth lens L .

Guide figure X makes both sections clear.

the normal condition and finally beyond to the incomplete diprosopus with three eyes and a fourth lens. The idea of arranging monsters in such a series including the normal is due to Prof. H. H. Wilder.

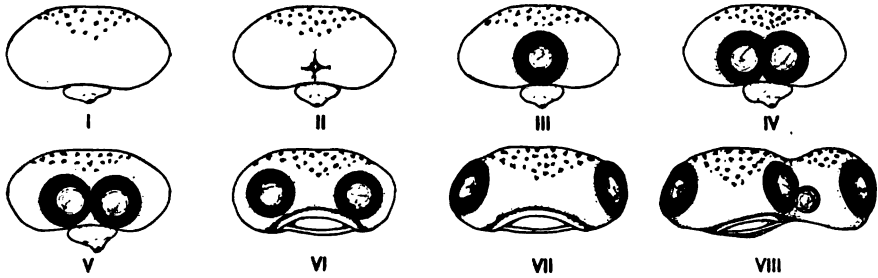


Fig. 58 Diagram of the various conditions shown by the "magnesium embryos" from entire absence of the cyclopean eye *I*, to deeply buried eye *II*, perfect single cyclopean eye *III*, double-eye, *IV*, two approximated eyes *V*, eyes unusually close together *VI*, normal *VII*, three eyes and fourth lens *VIII*.

The normal is a mean from which different degrees of abnormalities are but greater or less deviations. It is possible to arrange almost any type of abnormality in such a series. Supernumerary arms or legs on one side might exist in various individuals in different numbers down to the single normal one; other specimens could be found showing degenerate or small arms and finally armless or legless individuals are known.

MORPHOLOGY OF MONSTRA MONOPHTHALMICA ASYMMETRICA

A brief description of the asymmetrical monophthalmica monsters in life has been given above, but their true nature and structural conditions are impossible to detect without sections. It is found that here again a continuous series exists, beginning with the ordinary two-eyed individual through all gradations to the complete disappearance of one eye.

The section through the middle of the eyes in a normal embryo of thirteen days old is illustrated in Fig. 59. The eyes, of course, are equal in size and alike differentiated structurally. In the salt solutions, however, many embryos occur with one eye perceptibly

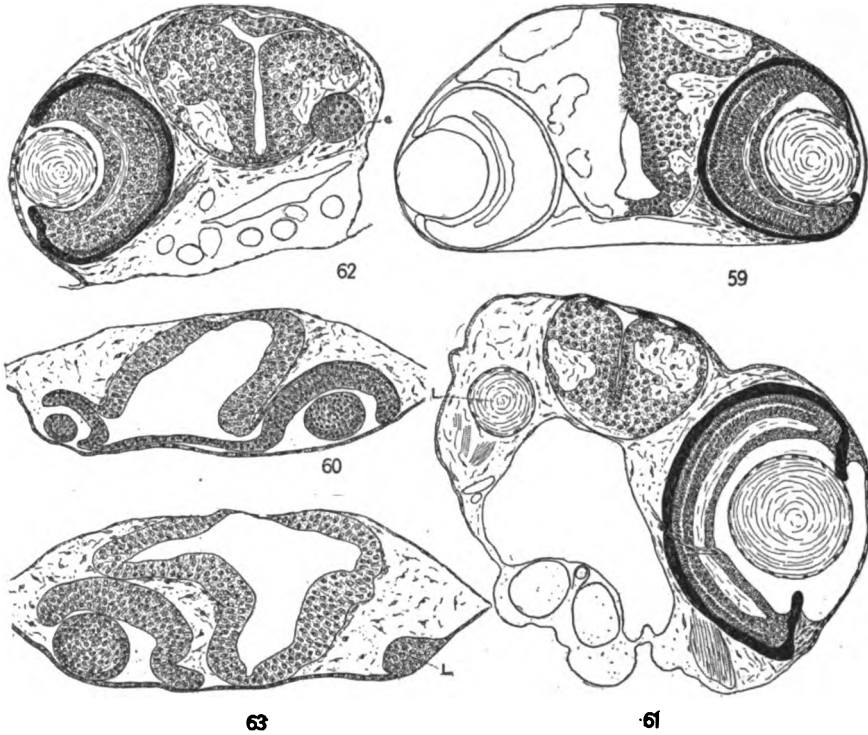
smaller than its mate. A section through the eyes of an embryo of this kind when seventy-six hours old is shown in Fig. 60. The left eye is decidedly smaller than the right and possesses a correspondingly small lens. From the comparative study of a number of individuals it may be safely stated that this difference in size between the two eyes will not be overcome later, nor on the other hand will the small eye degenerate or disappear. The embryo will hatch with its eyes in dissimilar conditions comparable to the state of things shown by this seventy-six hour stage. The brain is normal and two nasal plates are present.

An embryo closely similar to the one just described was sectioned after hatching. Its large eye appears as in Fig. 61. More anterior sections show a small eye looking forward with a somewhat protruding lens in its cup. Behind this small eye is another lens lying free in the ectoderm (shown in Fig. 61). This lens is perfectly differentiated and appears to have arisen independently.

A further reduction of the eye is shown by Fig. 62. In this thirteen day embryo the left eye is perfect and the right is represented by a small cellular mass lying close against the brain. The lens of the right side is entirely wanting. In life the head was slightly one-sided, obviously on account of the asymmetrical eye development; no indication of the cellular mass could be detected and the embryo seemed truly one-eyed.

A section of another seventy-six hour individual which in life also seemed to be one-eyed is illustrated by Fig. 63. The brain is normal and almost bilaterally symmetrical, an ordinary left eye exists but there is not the trace of an indication of the right optic cup. An ectodermal thickening represents the right lens in process of formation in the position that it would typically occupy. This lens anlage must have arisen independently of a stimulus from an optic cup and is well removed from the brain, so that no direct stimulus from that source can be responsible for its appearance.

Other one-eyed individuals showed complete absence of all parts of the second eye, the lens as well as the optic cup failing to arise. The occurrence in the Mg solutions of these one-eyed embryos as well as the cyclopean embryos suggests that the chem-



Monstra monophthalmica asymmetrica

Fig. 59 Section through eyes of normal thirteen day embryo.

Fig. 60 Section of seventy-six hour embryo with one normal and one small eye and perfect brain.

Fig. 61 Section of the normal eye of a hatched embryo; a small eye with a lens is situated more anteriorly on the other side and behind this is a third lens, *L*, shown in the figure on the left side.

Fig. 62 Section of normal eye in thirteen day embryo, the other eye is represented by the cellular mass, *e*, close against the brain.

Fig. 63 Section of normal eye in seventy-six hour embryo, the brain is bilateral and perfect, but no indication exists of the right optic cup although the ectoderm of that side has formed a lens thickening *L*.

ical influence exerts a peculiar inhibition of that process of out-pushing or separation by which the optic vesicles arise. Such an idea will be more fully considered in the general discussion given below.

The unequal eyes may possibly result from an unequal allotment of eye material to one side or the other. A major portion might go to the right side and a minor part to the left, or the entire eye anlagen might by chance occur on one side. This in a sense would be lateral cyclopia. Such reasoning is of course purely hypothetical.

INDEPENDENT ORIGIN AND SELF-DIFFERENTIATION OF THE CRYSTALLINE LENS

Spemann ('01), Lewis ('04), and others have concluded from experiments on amphibian embryos that there is no localization of lens-forming material in any given area of the ectoderm. They further held that the formation of a lens is dependent upon a stimulation of the ectoderm through contact with the optic-vesicle or cup. Spemann ('05) in discussing the question of the self-differentiating power of the lens concluded from a consideration of Schaper's ('04) experiments on the frog that the lens is not capable of self-differentiation, but that a continued influence or contact of the optic-cup is necessary to cause the lens-plate or lens-bud to develop into a typical lens. LeCron ('07) has recently shown that the lens in *Amblystoma* is not self-differentiating. I ('07d) found in embryos of the blind Myxinoid, *Bdellostoma stouti*, that a lens-thickening formed in early stages while the optic-vesicles were near the ectoderm. During development the optic cup becomes distantly removed from the ectoderm and the lens-plate disappears as if it were unable to continue development independently of the optic cup contact.

On the other hand Mencl ('03) has claimed that the lens in *Salmo salar* is at times formed independently of the optic cup influence and Spemann ('07) has recently modified his attitude. Spemann finds that in a certain species of frog, *Rana esculenta*, the lens may arise independently of the optic cup. This lens also

continues to develop and differentiates typical **fibers**. Most conclusive evidence favoring the independent origin and self-differentiation of the lens is furnished by the *Fundulus* embryos now under consideration.

Attention has been called repeatedly to the occurrence of lenses having no connection with other optical parts. It may be well at this time to summarize these cases which clearly show that in *Fundulus* the lens may arise independently and continue its development and differentiation.

Fig. 63 illustrates the budding off of the lens from ectoderm on the side of the head which lacks entirely an optic cup. Fig. 61 shows a lens fully differentiated though lying freely in the mesenchyme of the head. It will be recalled that this is a supernumerary lens; the large and small eyes of the embryo both possess lenses. An optic cup can not be responsible for this third lens. Fig. 57 of the incomplete diprosopus shows the fourth lens of the double head entirely outside the optic cup of the third eye which possesses a lens. Figs. 54 and 55 show two lenses in an embryo that possessed no trace of an optic cup. Fig. 53 indicates a lens in its usual position but no optic cup is present. In Fig. 45 a tiny lens is found in front of a cyclopean eye which possesses its own lens. Many other similar illustrations could be given.

No one could hold that this indiscriminate collection of lenses, all of which are entirely isolated from any connection with optic cups or other eye parts, as well as in nearly all cases from the brain itself has arisen through direct stimuli derived from the optic cups. It is also evident that the lens after its formation continues to self-differentiate.

It seems to me that in *Fundulus* the case is clearly proven that lens formation does not depend upon a direct stimulus from the optic cup. Such a dependence as advanced by Lewis ('04) for the frog is not, therefore, of universal application, nor is the view tenable that the differentiation of the lens depends upon a continued stimulus from the optic cup.

DISCUSSION AND CONCLUSIONS

The foregoing facts furnish important information as to the cause and manner of development of cyclopia, and the facts bear directly on previous ideas concerning this subject.

By treating the fish eggs with magnesium solutions, it is conclusively shown that the experimenter has the power without mechanically injuring the egg or embryo to cause what would have been a two-eyed individual to become a cyclopean monster. This undoubtedly is a case of the occurrence of cyclopia through the action of external influences on the developing egg. I conclude, then, that cyclopia does not necessarily result from germinal variations, but I make no claim that it may never arise in such a way. On the contrary, there is no reason why cyclopia should not occur through germinal variations as readily as does any other new feature. The fact that mammalian cyclopean monsters do not survive, or even if it be proven that the free-swimming cyclopean fish are incapable of living or reproducing, does not argue against the possibility that cyclopia may in cases be due to germinal variation. Such a statement is emphasized by a case I ('07c) recently recorded. In a flock of sheep in North Carolina two entirely legless lambs appeared in the spring of 1907. Again in 1908 two other similar lambs have occurred, one being the offspring of a mother which had previously borne a legless individual. These lambs were unable to feed without assistance and in nature would doubtless have died shortly after birth, but their peculiar occurrence in this flock is very probably due to germinal variations, either within the mother or father, or both. Students of inheritance consider sports to be due to germinal variations and the ability of such sports to survive depends merely on their adaptations to the surroundings and not in the least on their manner of origin. No reason can be given why a cyclopean individual might not occur as a sport due to sudden germinal variations. From the experiments contained in the present paper, however, it may be emphatically affirmed that cyclopia is *not always* due to germinal origin.

Spemann ('04) through an ingenious method of experiment, produced double-headed Triton embryos which exhibited various

degrees of cyclopia. The eggs of this salamander when constricted about the periphery of the first plane of cleavage with a fiber-like ligature gave monsters with two equal heads. When the ligature was oblique with reference to this plane one of the heads was cyclopean to a greater or less degree. Spemann thought the defective head due to the loss of the anlagen of certain parts, consequently these parts never began development and organs situated lateral to them developed in contact from the start. In other words parts between the eye anlagen fail to form and thus the anlagen come in contact and so develop from the beginning. This explanation is of course entirely speculative, but it is supported in a manner by experiments which according to Mall ('08) Lewis has performed on the fish embryo. Mall states that Lewis found by pricking the extreme anterior end of the embryonic shield in *Fundulus* eggs that many of the eggs develop into cyclops embryos. It was found in some that the prick had destroyed the "nose" only. "This experiment shows conclusively that it is the absence of tissues between the eye anlagen that allows them to come together and unite."

The above explanation no doubt holds for some cases of cyclopia produced by cutting or pricking; there it is evident that tissue is destroyed and the destruction of median tissue may cause the regions containing the eye anlagen to unite. It is difficult to apply this explanation to all cases. In the "Magnesium embryos," why should tissue between the eyes fail to form and not other tissues; why are the nasal pits united in some cyclops and separate in others? A close microscopic examination of the brain floors in cyclopean and two-eyed embryos shows no absence of recognizable parts in the former. The monstra monophthalmica asymmetrica are also to be explained; here one eye in some cases fails to come off from the brain. Is this due to the absence of its early anlage? The very small cyclopean eye sometimes buried deeply in the head, and the eye shown in Fig. 38 which is partly inclosed within the brain, as well as the entire absence of an eye, suggest another explanation that may apply to all cases in the magnesium solutions.

The small eyes close together, cyclopia in various degrees, the

imperfect formation or absence of one eye and entire absence of eyes are all conditions common to the magnesium solutions and very rare or never occurring in other solutions, nor in the hundreds of eggs observed developing in sea-water. The conditions are, therefore, probably due to a common cause, and I suggest hypothetically that this cause is an inhibitory or anæsthetic effect of the magnesium on the process of outpushing and separation of the optic vesicles. Magnesium exerts a decidedly anæsthetic effect upon both vertebrate and invertebrate animals and is an inhibitor of muscular activity. It might possibly inhibit the giving off of the optic vesicles or prevent their separation in the brain, so that both might come off together as in cyclopia, and it might have caused the eye in Fig. 38 to be arrested when only halfway separated from the brain; the absence of one eye and complete absence of eyes would be perfect inhibition. It is necessary to find a definite point in the strength of the solutions in order to obtain the proper amount of inhibition for many weaker eggs are killed during early stages.

The strongest argument against such an hypothesis is the fact that Mg in distilled water solutions fails to cause cyclopia, whereas its anæsthetic or inhibiting powers should be most active in such a solution.

Dareste's ('91) idea that cyclopia is caused by a closed brain or the failure of the anterior vesicle to develop is unsupported, since in Triton with the hollow-brain tube present Spemann finds that the defect occurs. In *Fundulus* the optic outpushings are normally given off while the brain is yet solid, so that according to Dareste all of these fish would be cyclopean in nature.

Schwalbe ('06) in his *Morphologie der Missbildungen des Menchen und der Tiere*, considers cyclopia to result from unusual pressure exerted during early stages of development which does not cause the lateral parts to grow together but prevents them from developing at all. This position is somewhat in accord with the hypothesis suggested above. If pressure prevents the growing apart laterally of the anlagen which normally require energy to accomplish their separation, then by anæsthetizing a part, one accomplishes practically the same thing as by applying pressure.

The part in anæsthesia lacks energy to grow out laterally, thus the two eye anlagen remain together in the floor of the brain and come off as one median vesicle either double or single, depending upon the extent of separation possible under the given degree of pressure or anæsthesia.

Mall ('08), in his recent memoir on the causes underlying the origin of human monsters, gives an excellent survey and discussion of the evidence furnished by experimental teratology. In the body of the paper is presented a strong case in favor of external influence during development as the chief cause of many monstrosities. Here we may consider only the discussion of cyclopia. The idea of fusion of the two eye vesicles during their development is advocated, but the present evidence is against this position and is in accord with Spemann's ('04) view of an early defective anlage. Mall also inclines toward the idea of the single brain as being primarily responsible for cyclopia, but it is shown by embryos considered here that cyclopia often accompanies perfectly bilateral and bilobed brains, neither does a retarded growth of the frontal process necessarily follow in cases of cyclopia.

Experiments uphold the statement "that every egg has in it the power to develop cyclops monsters." The germinal theories of cyclopia are shown by the experiments to be unnecessary as explanations of its cause. The possibility of its occurrence through germinal variations, though to my mind extremely slight, is not entirely excluded by experiments. The experiments conclusively show the origin of cyclopia through external influences.

Much could be said pro and con regarding the significant nature of the cyclopean fish embryos as a specific response to a definite chemical environment. The suggestion is evident, though highly hypothetical, that cyclopia in man and mammals might be due to a similar chemical cause, an excess of Mg salts in either the mother's blood or the amniotic fluid surrounding the developing embryo.

The Magnesium embryo is as typical of these Mg solutions as is the now classic lithium larva of the sea urchin produced by Herbst ('92, '93) in his Li solutions, or Morgan's ('04) lithium frog embryos produced in a similar way. They all tend to show that dif-

ferent chemical conditions may each induce by their actions a specific type of larva from a given variety of egg.

SUMMARY

1 The eggs of the fish, *Fundulus heteroclitus*, give rise to a large percentage of cyclopean embryos when subjected during their development to solutions of magnesium salts in sea-water. Similar results follow if the eggs are placed in the solutions either before cleavage or when in the two or early four-cell stages, later stages were not tried. This is the first instance of repeatedly causing, by the use of chemical substances, vertebrate monstrosities such as are known in nature.

2 The peculiar embryos with the median cyclopean eye are able to hatch. Many of them swim about in a perfectly normal manner, darting back and forth to avoid objects placed in their field of vision as readily as do two-eyed individuals.

3 The cyclopean fish is exactly comparable to the monstrous cyclops of man and other mammals. Both have a median eye either double or single in its structure. The nose in the mammalian cyclops is a single proboscis-like mass above the eye. The nasal pits in the "Magnesium embryos" are sometimes united and sometimes separate, but the mouth hangs ventrally as a proboscis-like organ strikingly suggesting in form the nose in mammalian cyclopia. The mouth of *Fundulus* normally occupies an extremely anterior position but in the cyclopean fish the eye has usurped this place, thus preventing the usual forward growth of the mouth elements and forcing them to remain ventrally as the proboscis-like mass. (See Figs. 25, 26, 27.) In cyclopean mammals a similar mechanical explanation accounts for the condition of the nose. The median eye obstructs the path of down-growth which passes normally between the eyes, and forces the nose to form above the eye as a proboscis on the forehead.

4 A study of more than 275 living cyclops monsters and of many of these in section shows all degrees in the defect. Eyes unusually close together, intimately approximated eyes, the double

eye in a median position, the single cyclopean eye, an extremely small anterior eye, a deeply buried ill-formed cyclopean eye, and finally an entire absence of the eye. The embryos exhibit these various degrees of the cyclopean defect from the earliest appearance of the optic outpushings, and in no case was cyclopia due to a union or fusion of the two eye components after they had originated distinctly.

5 A second type of monster designated as *Monstrum monophthalmicum asymmetricum*, the monster with one asymmetrical eye, was also common in the magnesium solutions. These individuals have one perfect eye of the normal pair but the other is either small, poorly represented or entirely absent. This condition is also present from the first appearance of eye structures and is not due to degeneration or arrest of development.

6 Both types of monsters often form lenses independently of the optic cup stimulus. These self-originating lenses are also capable of perfect self-differentiation, forming lens fibers and appearing as transparent crystalline bodies. Such facts oppose the idea that the lens during its origin and development is in a dependent relationship with the optic cup, and show this view not to be of universal application.

7 The experiments conclusively prove that eggs may be induced to develop into cyclopean monsters by external influences. These influences do not mechanically injure or destroy certain eye regions as does cutting or pricking. It follows, therefore, that cyclopean monsters appearing in nature are not necessarily due to germinal variations, but are far more likely the result of some unusual external influence during development.

8 The occurrence of the various eye monstrosities shown by embryos which develop in magnesium solutions are all probably due to a common cause and I suggest the following hypothetically: Magnesium which possesses a decidedly anæsthetic effect on most animals and is inhibitory in its influences on muscular activity may retard through degrees of anæsthesia the optic outpushings in *Fundulus* embryos and thus account for the total absence of eyes, small eyes, eyes which failed to develop energy necessary for their normal separation and the other unusual conditions which

have been considered in detail in the present article. This view, of course, is hypothetical and objections to it are recognized.

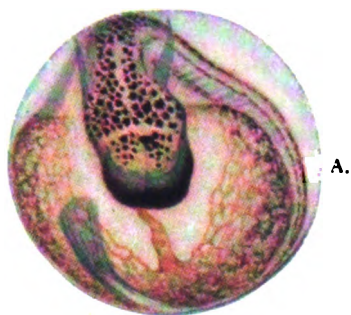
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LITERATURE CITED

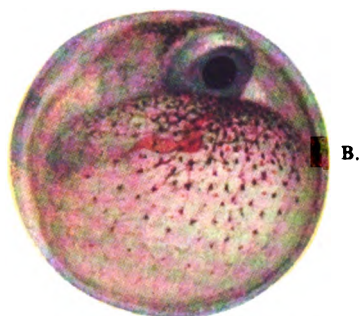
- DARESTE, C. '91—Recherches sur la production artificielle des monstruosités ou essais de tératogénie expérimentale. pp. 366-383, Paris.
- DOHRN, A. '75—Der Ursprung der Wirbelthiere und das Princip des Funktionswechsels. Leipzig, pp. 1-87.
- HERBST, C. '92—Experimentelle Untersuchungen über den Einfluss der veränderten chemischen Zusammensetzung des umgebenden Mediums auf die Entwicklung der Thiere. I. Theil. Zeitsch. f. wissensch. Zool. iv, pp. 446-518.
- '93—Experimentelle Untersuchungen. II. Theil. Mittheil. aus der Zool. Station zu Neapel, xi, pp. 136-220.
- '01—Formative Reize in der thierischen Ontogenese. Ein Beitrag zum Verständniss der thierischen Embryonalentwicklung. Leipzig.
- LECROON, W. L. '07—Experiments on the Origin and Differentiation of the Lens in Amblystoma. Am. Journ. Anat., vi, pp. 245-258.
- LEWIS, W. H. '04—Experimental Studies on the Development of the Eye in Amphibia. I. On the Origin of the Lens. *Rana palustris*. Am. Journ. Anat., iii, pp. 505-536.
- MALL, F. P. '08—A Study of the Causes Underlying the Origin of Human Monsters. Journ. Morph., xix, pp. 1-361.
- MENCL, E. '03—Ein Fall von beiderseitiger Augenlinsenausbildung während der Abwesenheit von Augenblasen. Arch. f. Entw'mech., xvi, pp. 328-339.
- MORGAN, T. H. '03—The Relation Between Normal and Abnormal Development of the Embryo of the Frog, as Determined by the Effects of Lithium Chlorid in Solution. Arch. f. Entw'mech., xvi, pp. 691-712.
- '06—Experiments with Frog's Eggs. Biol. Bull., xi, pp. 71-92.
- SCHAPER, A. '04—Ueber einige Fälle atypischer Linsenentwicklung unter abnormen Bedingungen. Anat. Anz., xxiv, pp. 305-326.
- SCHWALBE, E. '06—Die morphologie der Missbildungen des Menschen und der Tiere. I. Theil. Allgemeine Missbildungslehre. Jena, pp. 1-230.

ARTIFICIALLY PRODUCED CYCLOPEAN FISH
CHARLES R. STOCKARD

PLATE I



A.



B.

RICHEOLI, *del*

THE JOURNAL OF EXPERIMENTAL ZOOLOGY, VOL. VI, NO. 2

- SPEMANN, H. '01—Ueber Correlationen in der Entwicklung des Auges. Verhandl. der Anat. Gesellsch., 1901.
- '04—Ueber experimentell erzeugte Doppelbildungen mit cyclopischem Defekt. Zool. Jahrb. Suppl. vii, pp. 429-470.
- '05—Ueber Linsenbildung nach experimenteller Entfernung der primären Linsenbildungszellen. Ausführlich: Zool. Ang., xxviii.
- '07—Neue Tatsachen zum Linsenproblem. Zool. Anz., xxxi, pp. 379-386.
- STOCKARD, C. R. '06—The Development of *Fundulus heteroclitus* in Solutions of Lithium Chlorid, with Appendix on its Development in Fresh Water. Journ. Exp. Zool., iii, pp. 99-120.
- '07a—The Artificial Production of a Single Median Cyclopean eye in the Fish Embryo by Means of Sea-water Solutions of Magnesium Chlorid. Arch. f. Entw'mech, xxiii, pp. 249-258.
- '07b—The Influence of External Factors, Chemical and Physical, on the Development of *Fundulus heteroclitus*. Journ. Exp. Zool., iv, pp. 165-201.
- '07c—A Peculiar Legless Sheep. Biol. Bull., xiii, pp. 288-290.
- '07d—The Embryonic History of the Lens in *Bdellostema Stouti* in Relation to Recent Experiments. Am. Journ. Anat., vi, pp. 511-515.
- '08—The Question of Cyclopia. Science, n.s., xxviii, pp. 455-456.
- WILDER, H. H. '08—The Morphology of *Cosmobia*. Am. Journ. Anat., viii, pp. 355-440.

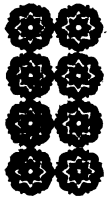
EXPLANATION OF PLATE I.

Fig. A Dorsal view of a cyclopean embryo in almost natural colors. The large antero-ventral eye shows a slight furrow indicating its double nature.

Fig. B The same embryo when the egg is rolled back towards the top of the page. A somewhat ventral view showing the single pupil and lens, the double condition of the eye is only indicated from above.

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